THE EFFECT OF PRONETHALOL ON OUABAIN-INDUCED ARRHYTHMIAS AND CARDIAC AUTOMATICITY AFTER RESERPINE, AND THE EFFECT OF OUABAIN ON THE β RECEPTOR BLOCKING ACTION OF PRONETHALOL

Ronald R. Tuttle

Department of Pharmacology and Therapeutics

University of Manitoba, Winnipeg 3, Canada

Nov. 1965: Awarded THE E.L. DREWRY MEMORIAL SCHOLARSHIP AND MEDAL for this thesis. (Silver Medal and $300).

Abstract

These experiments show that the antagonism of ouabain-induced arrhythmias by pronethalol is not due to blockade of the cardiac action of endogenous catecholamines. Depletion of catecholamines by pretreatment with reserpine did not impair the ability of pronethalol to restore sinus rhythm. Moreover, the antiarrhythmic effect of pronethalol was not temporally correlated with β receptor blockade. Single intravenous injections of pronethalol suppressed the arrhythmia for only brief periods, but blocked the chronotropic action of isoproterenol for several hours. In order to maintain sinus rhythm in a dog poisoned with ouabain pronethalol had to be given continuously by intravenous infusion.

The results indicate that pronethalol does not act by an unspecific depressant action similar to quinidine. In reserpine-pretreated dogs pronethalol (in concentrations adequate to reverse ouabain-induced arrhythmias) stimulated rather than depressed the heart. It caused an increase in the normal sinus rate, and an increase in the frequency of beats originating from subatrial pacemakers which were elicited by vagal stimulation.

It is suggested that pronethalol and ouabain are mutually antagonistic in the heart. Evidence to support this was gained from experiments showing that ouabain antagonized the β receptor blocking action of pronethalol. Ouabain partially restored the chronotropic action of isoproterenol in dogs previously treated with pronethalol. In the absence of pronethalol ouabain inhibited the chronotropic response to isoproterenol.

Introduction

Dichloroisoproterenol (DCI) and pronethalol were shown to selectively block the inhibitory response in smooth muscle and the excitatory response in cardiac muscle to adrenergic drugs (Powell and Slater, 1958; Moran and Perkins, 1958; Black and Stephenson, 1962; Donald et al., 1964; Koch-Weser, 1964). For this reason DCI and pronethalol are classed as β receptor blocking agents according to the hypothesis of Ahlquist (1948). In addition to blocking the positive chronotropic and inotropic action of adrenergic drugs, DCI and pronethalol antagonize adrenergically-induced arrhythmias (Gilbert et al., 1959; Somani and Lum, 1965; Lucchesi, 1965).

Lucchesi and Hardman (1961) showed that DCI antagonized ouabain- and acetylstrophanthidin-induced arrhythmias in dogs and isolated rabbit hearts. Abatement of the arrhythmias, however, was not correlated with β receptor blockade. The arrhythmias recurred at a time when β receptor blockade was still present, and structural analogues of DCI which failed to block β receptors also antagonized the arrhythmias. Similar studies by Lucchesi (1964 and 1965) showed that pronethalol was like DCI in that it suppressed arrhythmias caused by cardiac glycosides and this could not readily be explained by β receptor blockade.

In a preliminary report (Tuttle and Innes, 1964) of the present work we concluded that the effect of pronethalol on these arrhythmias was not due to blockade of the cardiac actions of endogenous catecholamines. This was based on the observation that pronethalol was as effective against ouabain-induced arrhythmias in dogs depleted of catecholamines by reserpine as it was in normal dogs. By a different experimental approach Somani and Lum (1965) have reached the same conclusion. They suggested that the antiarrhythmic effect of pronethalol was due to an unspecific "quinidine-like" action. The present results, however, do not support this suggestion.

Methods

Mongrel dogs of either sex were anesthetized with pentobarbital sodium i.v. (35 mg/kg for untreated animals and 20 mg/kg for reserpine-pretreated animals), and maintained on positive pressure respiration with room air. The vagus nerves were cut at the level of the larynx in all dogs. Femoral arterial pressure was measured through a polyethylene catheter filled with 4% heparin solution and connected to a Statham P-23 pressure transducer. The arterial pressure and the Lead II ECG were recorded on a Grass Polygraph. The heart rate was counted from the ECG. Rectal temperature was kept at 37°C by a heating lamp.

In one group of reserpine-pretreated dogs a cardiac rhythm originating from a subatrial pacemaker was elicited by stimulating the vagus nerve. The peripheral stump of the cut right vagus was placed across the contact points of a bipolar platinum electrode and carefully positioned in the neck to avoid stretching the nerve. Liquid petrolatum (U.S.P.) was poured into the neck incision so that it formed a pool around the nerve and the end of the electrode. Square wave pulses 1 millisecond in duration were supplied by a Grass SD-5 Stimulator. Threshold voltage was determined in each dog by briefly stimulating the nerve at a frequency of 20 pulses/second and gradually increasing the voltage (starting at 1 volt) until cardiac slowing was seen on the ECG. To elicit a subatrial rhythm the vagus was stimulated for 90 seconds at twice the threshold voltage and at a frequency of 20 pulses/second. Within the first 60 seconds of stimulation there was a marked bradycardia followed by a period (5-40 sec) of cardiac arrest; then vagal escape occurred and the P wave was absent from the ensuing rhythm. The absence of the P wave was taken as evidence of a subatrial pacemaker. The frequency of the subatrial rhythm was determined by counting every beat during the last 30 seconds of stimulation. When vagal stimulation was stopped

at the end of the 90 second period normal sinus rhythm immediately reappeared.

All drugs were prepared daily in saline dilutions from stock solutions and injected intravenously. Reserpine was dissolved in a mixture of dilute acetic acid and propylene glycol and injected (0.5 mg/kg) intraperitoneally 48 and 24 hours before the experiment. Paasonen and Krayer (1958) have shown that this treatment depletes the dog heart of all but negligible amounts of norepinephrine and epinephrine. Doses of isoproterenol are expressed as the weight of the free base, and doses of pronethalol as the weight of the hydrochloride.

Tests for statistical significance within groups (experiments in which each animal served as its own control) were done by the "t" test for paired data. Comparisons between groups were made by Student's "t" test. Values for P were obtained from a one tailed "t" table. The means reported are given with their standard errors.

Results

Ouabain (85 μg/kg) was given to five dogs. With 5 to 10 minutes ventricular tachycardia appeared in three of the dogs, one developed nodal rhythm, and one developed bigeminal rhythm. Pronethalol (5 mg/kg) was injected 10 to 20 minutes after the appearance of the arrhythmia. In each dog the abnormal rhythm was converted to sinus rhythm within 30 seconds of the injection. However, sinus rhythm was brief. The arrhythmias recurred 1 to 9 minutes after giving pronethalol. Several (3-5) more injections of pronethalol (5 mg/kg) were given over the next 2 to 5 hours. Each time the arrhythmia was converted to sinus rhythm, but in no case did it persist for longer than 11 minutes. Ouabain (85 μg/kg) was given to four other dogs, but pronethalol was withheld. In these animals the arrhythmias lasted from 3 to 5 hours.

The administration of ouabain (85 μg/kg) always resulted in a prolonged rise in arterial pressure (60-125 mm Hg). The arrhythmias occurred within 5 to 10 minutes of giving ouabain and the pressor response was still present at this time. Reversal of the arrhythmias by pronethalol was always preceded by a sharp fall in arterial pressure (20-60 mm Hg). To determine whether the antiarrhythmic effect of pronethalol was due to its effect on the blood pressure, two different procedures were carried out on five dogs after inducing arrhythmias with ouabain (85 μg/kg). In three dogs pronethalol was withheld and the arterial pressure was rapidly lowered (50 mm Hg in 15 sec) by hemorrhage. In the other two dogs pronethalol (5 mg/kg) was injected 10 minutes after the appearance of the arrhythmia, but the depressor response was prevented by giving an intra-arterial infusion of blood. Hemorrhage had no effect on the arrhythmia in the first three dogs, and pronethalol still reversed the arrhythmia in the last two dogs.

Since sinus rhythm was maintained for only brief periods after reversal

of the arrhythmias, 4 experiments were done to determine the duration of catecholamine blockade caused by pronethalol (5 mg/kg). Chronotropic responses to isoproterenol (2 μg/kg) given at 15 minute intervals were recorded before and for 4 hours after giving pronethalol (5 mg/kg). The cardioaccelerator responses to isoproterenol were reduced by an average of 91% (S.E. ± 2), 68% (S.E. ± 11), 61% (S.E. ± 19), and 54% (S.E. ± 21) at 1,2,3, and 4 hours respectively after pronethalol. Clearly the transient antiarrhythmic effect of pronethalol was not in keeping with its sustained blocking action.

Four experiments were done to determine whether sinus rhythm could be maintained by a constant intravenous infusion of pronethalol. Ventricular tachycardia appeared within 2 to 10 minutes of giving ouabain (85 μg/kg). A single injection of pronethalol (5 mg/kg) reversed the arrhythmia. As soon as sinus rhythm was restored an intravenous infusion of pronethalol (200 μg/kg/min) was started. The infusion kept each dog in sinus rhythm for the duration of the experiment (4 hr).

The effect of pronethalol on ouabain-induced arrhythmias in dogs pretreated with reserpine. It was reasoned that if reversal of the arrhythmias by pronethalol depended on its ability to block the cardiac action of endogenous catecholamines, it should be ineffective in animals depleted of catecholamines. Thirteen dogs were pretreated with reserpine (0.5 mg/kg) 48 and 24 hours before their use. Within 5 to 17 minutes of giving ouabain (85 μg/kg) ventricular tachycardia developed in each dog. The arrhythmia was treated with pronethalol 15 minutes after its beginning in nine dogs. In four dogs pronethalol was withheld. Five of the nine treated dogs were given single injections of pronethalol (5 mg/kg); the other four were treated with infusions of pronethalol (200 μg/kg/min).

Single injections of pronethalol converted the arrhythmia to sinus rhythm within 30 seconds on each of several (3-5) trials in all five dogs. In two of

these experiments sinus rhythm was only temporary (1-24 min), but in the other three sinus rhythm persisted after the third trial until the experiments were ended 1 to 3 hours later. In three of the four dogs treated with infusions of pronethalol the arrhythmias were reversed within 3 to 13 minutes and sinus rhythm was maintained for 2 hours, after which the infusion was stopped and the experiment ended. In the fourth dog pronethalol (200 μg/kg/min) failed to reverse the arrhythmia; after 1 hour the infusion rate was doubled and the arrhythmia was immediately reversed, and sinus rhythm was maintained until the experiment was terminated 1 hour later.

Two of the four dogs not given pronethalol died; one at 39 minutes and one at 46 minutes after the injection of ouabain. In the other two ventricular tachycardia persisted for over 3 hours before the experiments were terminated.

The effect of pronethalol on cardiac automaticity in reserpine-pretreated dogs. It was of interest to determine whether the antiarrhythmic effect of pronethalol could be explained by an unspecific depressant effect on cardiac automaticity independent of its ability to block the cardiac actions of catecholamines. Accordingly 12 experiments were done on dogs pretreated with reserpine. The normal sinus rate and the frequency of beats originating from a pacemaker below the atria were taken as an index of cardiac automaticity. Subatrial pacemakers were elicited by stimulating the right vagus.

In four out of four dogs pronethalol (200 μg/kg/min) increased the sinus rate. The average maximum increase in heart rate was 30.2 $\pm$ 5.2 beats/min and occurred within 3 to 9 minutes after the start of the infusion. The heart rate remained above the pre-infusion level throughout the period of infusion (30-45 min) (Fig 1).

In four other dogs the frequency of the subatrial rhythm during vagal escape was measured before and 10 minutes after beginning the pronethalol

(200 μg/kg/min) infusion. A period of 10 minutes of infusion was chosen because this was about the time required in the earlier experiments for a similar infusion of pronethalol to reverse ouabain-induced arrhythmias. In all four dogs pronethalol increased the frequency of beats without restoring sinus rhythm (Fig 2). The average increase was 14.5 ± 1.2 beats/min ($P < 0.01$). In another four dogs pronethalol was withheld and two measurements of the subatrial rate were made 10 minutes apart. There was no significant change between the first and second measurement (1.25 ± 1.3 beats/min). There was a statistically significant difference between the dogs given pronethalol and the latter control group ($P < 0.01$).

The effect of ouabain on β receptor blockade. Experiments were done on 16 normal dogs to determine whether ouabain influenced the β receptor blocking action of pronethalol. The experiments were divided in four groups with four dogs in each group. Inhibition of the cardioaccelerator action of isoproterenol was used as an index of β receptor blockade. In the first two groups five injections of isoproterenol (2 μg/kg) were given at 10 minute intervals before and 15 minutes after giving either ouabain (50 μg/kg) or saline (5 ml). The mean increase in heart rate caused by the first five injections was compared to the mean increase caused by the second five injections. The experimental procedure was the same for the second two groups of animals except that pronethalol (20 mg/kg infused over 60 min) was given before starting the isoproterenol tests.

The notable finding in these experiments was that ouabain inhibited the cardioaccelerator action of isoproterenol in the absence of β receptor blockade, but in the presence of the pronethalol-produced blockade ouabain partially restored the action of isoproterenol (Tables 1 & 2). In the group without pronethalol ouabain reduced the response to isoproterenol by an average 11.5 ± 3.4 beats/min (Table 1, group 1). In the control group in which saline was given instead of

ouabain there was no significant change in the response (Table 1, group 2).

In the second two groups in which pronethalol was given first, there was an increase in the response to isoproterenol after giving ouabain and after giving saline. However, the increase after giving ouabain was an average of 19.5 $\pm$ 2.6 beats/min (Table 2, group 3), while after giving saline the increase averaged only 10.7 $\pm$ 2.0 beats/min (Table 2, group 4). The difference between these two groups was statistically significant ($P < 0.025$). The increased responsiveness of the heart to isoproterenol after giving saline indicated that there was some recovery from β receptor blockade during the course of the experiments. However, the enhanced action of isoproterenol after ouabain was too great to be explained on this basis. Therefore it was concluded that ouabain lessened the degree of β receptor blockade.

There was no significant change in the basal heart rate (the heart rate just before each injection of isoproterenol) between the first and second five injections of isoproterenol in any of the groups.

Discussion

Interpretation of the antiarrhythmic effect of pronethalol is complicated by its ability to block the cardiac action of endogenous catecholamines. For this reason the present experiments were done on animals pretreated with reserpine as well as on untreated animals. The results show that pronethalol is as effective against ouabain-induced arrhythmias in dogs depleted of catecholamines as it is in normal dogs. Clearly then, the antagonism of these arrhythmias by pronethalol does not rely on blockade of endogenous catecholamines. By a different experimental approach Lucchesi (1965) and Somani and Lum (1965) reached the same conclusion. The latter authors found that N-isopropyl-p-ethanolamine blocked the chronotropic and inotropic actions of epinephrine and isoproterenol, but did not antagonize arrhythmias caused by ouabain. Conversely, Lucchesi showed that the dextro isomer of pronethalol reversed ouabain-induced arrhythmias, but was ineffective as a β blocker. Our experiments support this conclusion by showing that a dose of pronethalol which caused sustained β receptor blockade only temporarily suppressed ouabain-induced arrhythmias. Similar results have been reported by Lucchesi (1964) and Somani and Lum (1965).

Since β receptor blockade fails to explain the ability of pronethalol to antagonize arrhythmias caused by cardiac glycosides, it has been suggested that the antagonism is due to an unspecific "quinidine-like" action (Somani and Lum, 1965). This hypothesis derives experimental support from the work of Sekiya and Vaughan Williams (1963) who demonstrated that pronethalol and quinidine had similar effects on intracellular cardiac potentials.

It is generally accepted that the principal direct action of quinidine on the heart is depression. Accordingly, if the antiarrhythmic effect of pronethalol relies on a "quinidine-like" action it should depress the heart in antiarrhythmic

concentrations. Such an action would oppose the increased ventricular automaticity caused by toxic doses of cardiac glycosides, and thus tend to suppress arrhythmia.

This explanation, however, is not supported by the present results. The direct action of pronethalol in dogs depleted of catecholamines was myocardial stimulation rather than depression. Pronethalol increased the frequency of beats originating from a subatrial pacemaker, and increased the normal sinus rate. These results are not surprising, for pronethalol has been shown previously to possess intrinsic sympathomimetic activity (Donald et al., 1964; Sekiya and Vaughan Williams, 1963). The significant point is that the dose of pronethalol used in these animals was the same as that used to reverse ouabain-induced arrhythmias in other animals similarly depleted of catecholamines. This indicates that pronethalol can exert its antiarrhythmic effect in a concentration which tends to stimulate rather than depress the heart. Therefore it would appear that this antiarrhythmic action is not due to an unspecific depression similar to quinidine. This view is consistent with observations made by Somani and Lum (1965) who found that pronethalol had no influence on arrhythmias caused by coronary ligation. In contrast, quinidine has been shown to be effective against arrhythmias caused by this method (Harris et al., 1951; Winbury and Hemmer, 1955; Clark and Cummings, 1956).

The above militates against both blockade of endogenous catecholamines and an unspecific "quinidine-like" action as the mechanism by which pronethalol antagonizes the arrhythmic action of cardiac glycosides. As an alternate hypothesis we suggest that there is a direct pharmacological antagonism between pronethalol and ouabain in the heart. If such a relationship exists, it might be expected that ouabain and pronethalol would be mutually antagonistic.

The present results are evidence of such an antagonism. The increased chronotropic response to isoproterenol after ouabain in animals previously treated

with pronethalol showed that ouabain lessened the degree of β receptor blockade. The enhanced action of isoproterenol could not be explained by sensitization of the heart by ouabain, since ouabain, in the absence of β receptor blockade, inhibited isoproterenol. The latter observation is similar to those made by Mendez et al. (1961) and Nadeau and James (1963) who showed that acetyldigitoxin and acetylstrophanthidin inhibited the chronotropic action of epinephrine. However, this is the first report of a cardiac glycoside antagonizing an adrenergic blocking agent.

References

Ahlquist, R.P.: A study of adrenotropic receptors. Amer. J. Physiol. 153: 586-600, 1948

Black, J.W. and Stephenson, J.S.: Pharmacology of a new β receptor blocking compound (Nethalide). Lancet 2: 311-314, 1962

Clark, B.B. and Cummings, J.R.: Arrhythmias following experimental coronary occlusion and their response to drugs. Ann. N.Y. Acad. Sci. 64: 543-551, 1956

Donald, D.E., Kvale, J. and Shepherd, J.T.: The effect of an adrenergic β receptor antagonist on the cardiovascular system of the dog. J. Pharmacol. 143: 344-349, 1964

Gilbert, J.L., Lange, G. and Brooks, C.McC.: Influence of sympathomimetic pressor drugs on arrhythmias caused by multiple stimuli. Circ. Res. 7: 417-423, 1959

Harris, A.S., Estandia, A., Ford, T.J. Jr., and Tillotson, R.F.: Quinidine lactate and gluconate in the suppression of ectopic ventricular tachycardias associated with myocardial infarction. Circulation 4: 522-533, 1951

Koch-Weser, J.: Direct and β adrenergic receptor blocking actions of nethalide on isolated heart muscle. J. Pharmacol. 146: 318-326, 1964

Lucchesi, B.R. and Hardman, H.F.: The influence of dichloroisoproterenol (DCI) and related compounds upon ouabain and acetylstrophanthidin induced cardiac arrhythmias. J. Pharmacol. 132: 372-381, 1961

Lucchesi, B.R.: The action of nethalide upon experimentally induced cardiac arrhythmias. J. Pharmacol. 145: 286-291, 1964

Lucchesi, B.R.: The effects of pronethalol and its dextro isomer upon experimental cardiac arrhythmias. J. Pharmacol. 148: 94-99, 1965

Mendez, C., Aceves, J. and Mendez, R.: Inhibition of adrenergic cardiac acceleration by cardiac glycosides. J. Pharmacol. 131: 191-198, 1961

Moran, N.C. and Perkins, M.E.: Blockade of adrenergic stimulation of the heart by 1-(3',4'-dichlorophenyl)-2-isopropylaminoethanol. J. Pharmacol. 122: 55, 1958

Nadeau, R.A. and James, T.N.: Antagonistic effects on the sinus node of acetylstrophanthidin and adrenergic stimulation. Circ. Res. 13: 388, 1963

Paasonen, M.K. and Krayer, O.: The release of norepinephrine from the mammalian heart by reserpine. J. Pharmacol. 122: 153-160, 1958

Powell, C.E. and Slater, I.H.: Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J. Pharmacol. 122: 480-486, 1958

Sekiya, A. and Vaughan Williams, E.M.: A comparison of the antifibrillatory actions and effects on intracellular cardiac potentials of pronethalol disopyrmanide and quinidine. Brit. J. Pharmacol. 21: 473-481, 1963

Somani, P. and Lum, B.K.B.: The antiarrhythmic actions of β adrenergic blocking agents. J. Pharmacol. 147: 194-204, 1965

Tuttle, R.R. and Innes, I.R.: Antagonism of ouabain-induced arrhythmias by nethalide. Pharmacologist 6: 165, 1964

Winbury, M.M. and Hemmer, M.L.: Action of quinidine, procaine amide, and other compounds on experimental atrial and ventricular arrhythmias in the dog. J. Pharmacol. 113: 402-413, 1955

TABLE 1

The effect of ouabain on the cardioaccelerator responses to isoproterenol

Group 1

Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2 μg/kg) before and after ouabain (50 μg/kg).

Dog No.	A Increase in H.R. before ouabain	B Increase in H.R. after ouabain	Difference (A-B)
1	59	40	19
2	75	67	8
3	76	62	14
4	60	55	5
Mean	67.5	56.0	11.5
			S.E. ± 3.43
			P < 0.05

Group 2

Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2.0 μg/kg) before and after saline.

Dog No.	A Increase in H.R. before saline	B Increase in H.R. after saline	Difference (A-B)
5	56	52	4
6	84	83	1
7	37	35	2
8	62	63	-1
Mean	59.7	58.2	1.5

TABLE 2

The effect of ouabain on the cardioaccelerator responses to isoproterenol after pronethalol (20 mg/kg)

Group 3

Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2.0 μg/kg) before and after ouabain (50 μg/kg)

Dog No.	A Increase in H.R. before ouabain	B Increase in H.R. after ouabain	Difference (B-A)
9	11	32	21
10	9	23	14
11	8	34	26
12	6	23	17
Mean	8.5	28.0	19.5
			S.E. ± 2.6
			P < 0.01

Group 4

Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2.0 μg/kg) before and after saline

Dog No.	A Increase in H.R. before saline	B Increase in H.R. after saline	Difference (B-A)
13	8	19	11
14	10	26	16
15	11	20	9
16	6	13	7
Mean	8.8	19.5	10.7
			S.E. ± 2.0
			P < 0.02

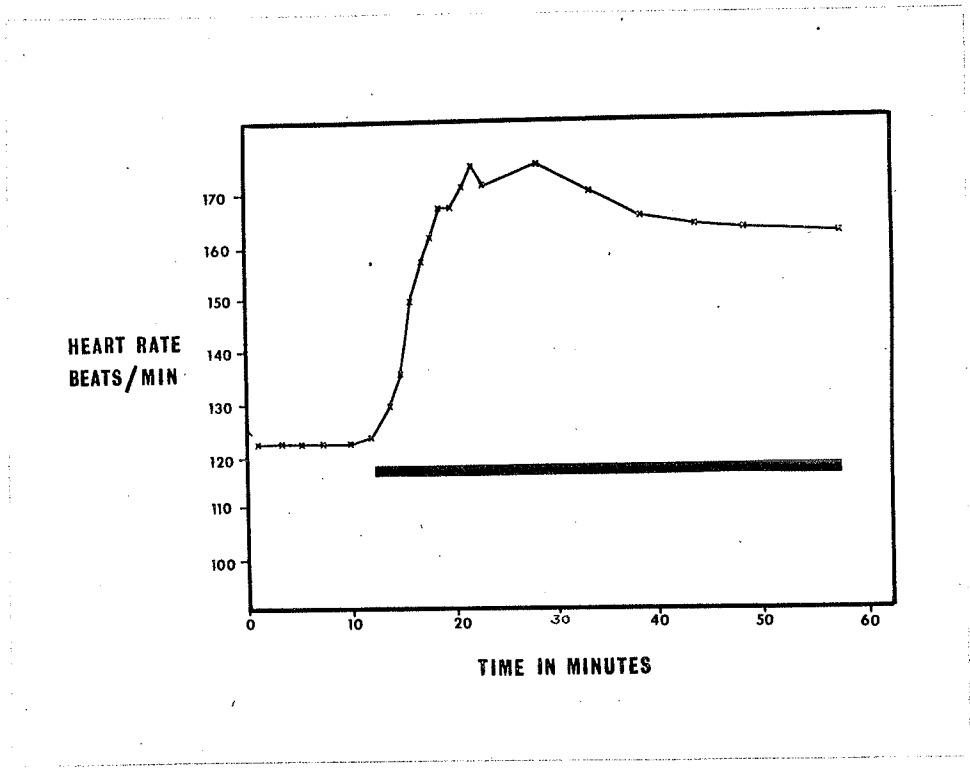

Figure 1 The effect of pronethalol on the heart rate of a reserpine-pretreated dog. Black bar indicates pronethalol (200 μg/kg/min) infusion i.v. Male dog 15 kg, Na pentobarbital (20 mg/kg), reserpine (0.5 mg/kg) i.p. 48 and 24 hours before the experiment, vagus nerves cut.

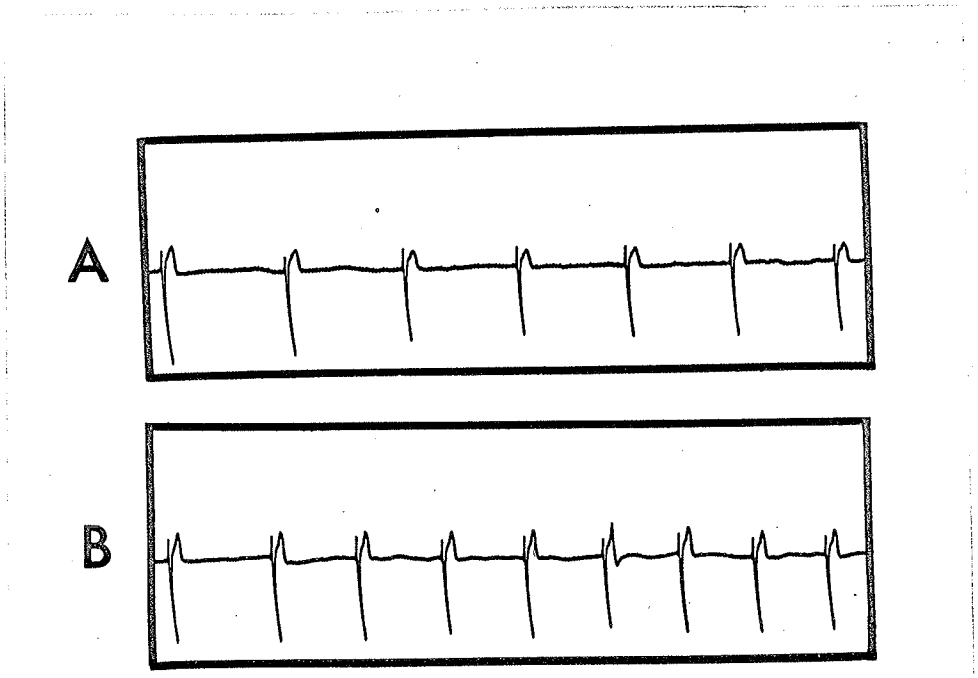

Figure 2 The effect of pronethalol on the rate of a subatrial rhythm elicited by vagal stimulation in a reserpine-pretreated dog. (A) Lead II ECG before pronethalol, rate is 60 beats/min. (B) Lead II ECG 10 minutes after start of a pronethalol (200 μg/kg/min) infusion i.v., rate is 83 beats/min. Paper speed is 25 mm/sec. Female dog 10 kg, Na pentobarbital (20 mg/kg), reserpine (0.5 mg/kg) i.p. 48 and 24 hours before the experiment, vagus nerves cut.