Short communications

Simultaneous measurement of atrial and ventricular pacemaker activity

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The isolated perfused rat heart with surgically-induced heart block was used to determine the effect of drugs on the activity of the atrial and ventricular pacemaker simultaneously. Complete atrio-ventricular dissociation was produced by tying a ligature around the Bundle of His under direct vision. Pacemaker rates remained stable for at least 2 hours. Perfusion of the hearts with quinidine produced a decrease in the rate of both the atrial and ventricular pacemaker. The ventricular pacemaker was more susceptible to the depressant action of quinidine than the atrial pacemaker.

The literature is replete with methods which have been used to induce cardiac arrhythmias experimentally. These procedures range from those employing methods to generate re-entry (Rosenbluth & García Ramos, 1947) to those causing increased automaticity by aconitine, catecholamines or digitalis (Scherf, Schaffer & Blumenfeld, 1953; Dawes, 1952; Roberts, Ito, Reilly & Cairoli, 1963). Still others have used combinations of procedures namely hydrocarbon and adrenaline (Garb & Chenoweth, 1948; Riker, Depierre, Roberts, Roy & Reilly, 1955), vagal stimulation and catecholamines (Roberts, Standaert, Kim & Riker, 1956; García de Jalón, Lastra & Serrano, 1969), electrical stimulation of the right atrium and acetylcholine (Burns, 1961) or electrical stimulation of the ventricle combined with exposure to isoprenaline (García de Jalón, Morantinos & Serrano, 1972). While each of these methods have provided useful information regarding the antiarrhythmic effects of drugs, with the exception of the methodology of Rosenbluth & García Ramos (1947), initiation of the rhythm disturbances depended on the action of a drug. Thus, the effect of a pharmacological agent in influencing such disturbances in rhythm may involve, at least in part, an antagonism between the drugs rather than a direct action on the basic physiological mechanisms involved in the genesis of the arrhythmia. To circumvent this problem, Nye & Roberts (1966) measured the effect of antiarrhythmic drugs on ventricular pacemaker activity by ligating the Bundle of His in isolated perfused cat hearts and recording simultaneously the spontaneous rates of the atrial and ventricular pacemakers. They reported that these rates remained stable for at least 3 to 4 h and thus it was possible to measure drug effects on the intrinsic automaticity of two pacemakers simultaneously. In the present report this technique has been extended to the rat since this animal is less costly and easier to handle than larger mammals.

Methods.—Charles River male rats 3 months of age weighing 275 to 314 g were used for this study. They were killed by decapitation and their hearts were immediately removed and placed in cold Krebs–Ringer solution (immersed in an ice bath) to bring about cardiac arrest. When arrest occurred a cannula was inserted into the aorta and the heart was then placed on a perfusion apparatus and perfused according to the method of Langendorff (Nye & Roberts, 1966). The heart was perfused with Krebs–Ringer solution bubbled with 95% O_2 and 5% CO_2; the temperature and pH of the solution were kept constant throughout the experiment at 37·5° C and 7·32, respectively. The Krebs–Ringer solution was perfused at a constant rate of 25 ml/minute. On perfusion with warm Krebs–Ringer solution the heart immediately resumed beating spontaneously.

The Krebs–Ringer solution contained (mM): Na^+ 146; K^+ 6·1; Ca^{++} 1·2; Mg^{++} 1·2; Cl^- 126; $SO_4^=$ 1·3; $H_2PO_4^+$ 1·2 and HCO_3^- 25·3.

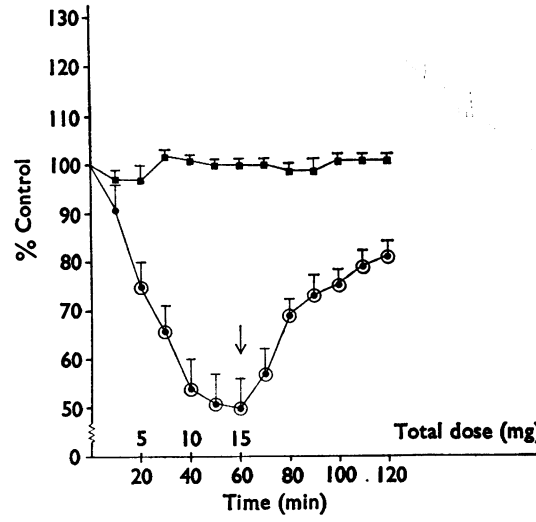
Stainless steel electrodes were attached to the left ventricle and to each atrium; the electrocardiogram was recorded on a polygraph. Pacemaker rates were determined from the electrocardiogram. The heart was allowed to equilibrate until its rate reached a constant level. This usually occurred within 30 min after perfusion was initiated. When the heart rate had stabilized the right atrium was opened at the margin near the right ventricle. A ligature (No. 5–0 cardiovascular suture) was placed around the area of the Bundle

of His with a small needle (3/8 taper T–2) and tied. Development of atrio-ventricular (A-V) block was confirmed by the electrocardiogram. If a block was not obtained due to mislocation of the tie, the procedure was repeated up to a maximum of 3 attempts, after which the preparation was discarded. In 80% of cases the method proved successful. When A-V block was established the preparation was allowed to stabilize for an additional 30 min ; at the end of this time interval the atrial and ventricular rates remained constant. In the control series Krebs–Ringer solution was perfused for 120 min and atrial and ventricular rates were recorded every 5 minutes. In the series in which the drug was studied, infusion of the agent began immediately after the equilibration period and was continued for 60 min and heart rates were recorded every 5 minutes. At the end of this period, the perfusion fluid was changed to normal Krebs–Ringer solution and recordings were made for another 60 min to determine the degree to which drug effects could be washed out. Quinidine sulphate was perfused in a concentration of 10 μg/ml ; the concentration is expressed in terms of the free base. The total dose of quinidine to which the heart was exposed was calculated by determining the total volume perfused and multiplying this volume by the concentration of quinidine. The dead space (180 ml) was taken into account in these calculations. Mean values and standard error (S.E.) of the mean are reported in the **Results** section.

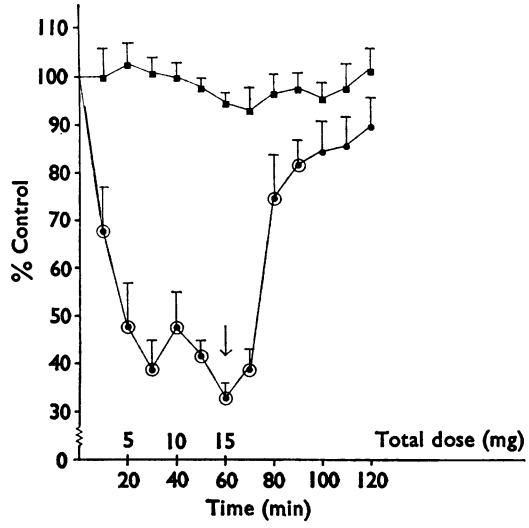
Results.—The normal pattern of activity as seen in Figs. 1a and b shows that rates of the atrial and ventricular pacemaker remained remarkably constant. The initial atrial rate was on the average 288 ± 10 beats/min and at the end of 2 h of perfusion it was 294 ± 10 beats/min ($P > 0{\cdot}05$). In the case of the ventricular pacemaker the initial rate was on the average 111 ± 12 beats/min and after 2 h of perfusion it was 114 ± 14 beats/min ($P > 0{\cdot}05$). It is interesting to note that the ratio of the atrial rate to the ventricular rate was approximately 2·6 while in the cat it is closer to 2 (Nye & Roberts, 1966).

Effect of quinidine

When the perfusion fluid was changed to that containing quinidine, a depression of both atrial and ventricular rates rapidly occurred. Maximum effects on the atrial and ventricular pacemaker occurred between 50–60 min after perfusion with the drug was initiated. The maximum effect was greater in the ventricular pacemaker than in the atrial pacemaker ; the ventricular rate was depressed approximately 67% while the atrial rate was depressed about 50%. In both cases when the perfusion fluid was changed to normal Krebs-Ringer solution the depressant effect of quinidine was rapidly reversed. In fact after 60 min,



(Fig. 1*a*)



(*b*)

FIG. 1. (a) Effect of quinidine on the rate of the atrial pacemaker in isolated rat hearts with heart block. Perfusion of quinidine was initiated at 0 time. The heart was perfused at a constant rate of 25 ml/minute. The arrow signals the time perfusion with quinidine was terminated. The initial atrial rate was 288 ± 10 beats/minute. (b) Effect of quinidine on the rate of the ventricular pacemaker in isolated rat hearts with heart block. Perfusion with quinidine as in (a). The initial ventricular rate was 111 ± 12 beats/minute. The vertical lines represent the S.E.M. ■——■=Controls. (In (a) $n=8$, in (b) $n=6$). ●——●=Quinidine-treated. (In (a) $n=8$, in (b) $n=6$). Circled values are significantly different from control ($P<0{\cdot}05$).

the atrial and ventricular rates had returned to 80% and 90% of control respectively.

Discussion.—The results of the present study demonstrate that the method of producing A-V block by ligation of the Bundle of His in isolated perfused rat hearts is a reliable and useful means of determining the effect of drugs or other interventions on the intrinsic automaticity of the atrial and ventricular pacemakers. The activity of these pacemakers remained remarkably constant for at least 2 h after A-V block and thus the effects of various procedures can be readily tested during this time interval. It is also important to note that it is possible with this method to determine the effect of drugs or other interventions on both atrial and ventricular pacemakers simultaneously and thus the relative susceptibility of each pacemaker to alterations in their environment can be evaluated. Another important feature of the method is that it involves measurement of intrinsic activity of these pacemakers and is not concerned with artificially-induced automaticity. Furthermore, drug effects can be readily washed out. Since the procedures can be done in the rat heart a relatively inexpensive and easy method is available for determining the effect of pharmacological interventions on pacemaker activity.

The results obtained with quinidine clearly indicate that the method will reveal actions of drugs which directly depress pacemaker function. Nye & Roberts (1966) reported that in cats the ventricular pacemaker was less susceptible to the negative chronotropic action of quinidine than the atrium, while in the present study the contrary seems to be the case. The reasons for the difference may involve species differences or perhaps other physiological parameters. Additional experimentation is needed before an explanation for this difference is uncovered.

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