Brit. J. Pharmacol. (1966), **26**, 473–481.

ACTION OF PROPRANOLOL ON THE ATRIO-VENTRICULAR NODE AND ON ITS RESPONSE TO ADRENALINE AND ISOPRENALINE

BY

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(*Received October* 29, 1965)

Propranolol has essentially the same pharmacological properties as pronethalol (Black, Crowther, Shanks, Smith & Dornhorst, 1964; Shanks, 1965). According to these authors its potency as an adrenergic-blocking agent in the heart is from ten to fifteen times that of pronethalol. Both compounds moderately reduce the spontaneous sinoatrial nodal frequency in anaesthetized or conscious mammals, and they block the sinus tachycardia produced by adrenaline and isoprenaline. These effects have recently been observed with propranolol in human subjects (Glover & Hutchison, 1965). Both compounds have been shown to block the positive inotropic action of isoprenaline in the dog ventricle (Black *et al.*, 1964).

In studies of cardioactive drugs, often little or no attention is paid to their effects on the atrioventricular node, despite the fact that serious disturbances of nodal function are not infrequently encountered in the clinic. Consider, for example, the passive nodal rhythms resulting from lesions or from drug intoxication, and nodal tachycardias of various aetiologies (digitalis intoxication, myocardial infarction, myocarditis, cardiac surgery). In view of the influence of the sympathetic nervous system upon the frequency and the refractory period of the atrioventricular node our interest in the theme of the present study becomes apparent. Accordingly, we have studied the action of propranolol on the automatism of the atrioventricular node, and upon its responses to adrenaline and isoprenaline. These responses include the effects of the catechol amines upon the frequency and the functional refractory period of the node. In addition, we have considered the influence of propranolol on the response of the atrial pressure to the catechol amines.

A preliminary report on this work was presented at the 7th Congress of the Mexican Society of Physiological Sciences, July, 1965.

METHODS

Experimental preparation. All of the experiments were conducted on dogs of either sex, ranging in weight from 12 to 24 kg, and anaesthetized with Dial-Ciba ("anaesthetic solution for animals") administered intraperitoneally in the amount of 0.7 ml./kg. In preliminary experiments pentobarbitone was used, but the repeated doses necessary to maintain an adequate level of anaesthesia reduced the arterial pressure and, on occasion, altered the automatism of the atrioventricular node to such a degree that the experimental results were discarded as unreliable.

After initiation of artificial ventilation the chest was opened in the midline, and bipolar recording electrodes were attached to the anterior surface of the right auricle and the right ventricle. Atrial and ventricular electrograms were recorded, together with a lead II electrocardiogram, on a Grass electroencephalograph. The vagus nerves were cut, but the sympathetic nerves were left intact. In a few preliminary experiments in which the thoracic sympathetic chains were also cut, the preparations deteriorated quickly, and the experimental results were discarded.

The sinoatrial node was destroyed by crushing the atrial wall from the lower margin of the superior vena cava almost to the coronary sinus. Functional exclusion of the sinoatrial node was more certain when the crushing clamp was left in place (Krayer, Arora & Meilman, 1955). In some experiments, in spite of extensive crushing of the node and surrounding tissue, it was not possible to achieve total exclusion of sinoatrial nodal activity.

Arterial pressure was routinely recorded on a kymograph by means of a mercury manometer from a femoral artery.

Functional refractory period of the atrioventricular transmission system. This was determined in the denervated heart according to the method of Krayer, Mandoki & Mendez (1951). Driving shocks of three times threshold intensity and 0.5 msec duration were applied through stimulating electrodes attached to the tip of the right auricular appendage. The driving frequency was selected to exceed the spontaneous frequency expected under the influence of the adrenergic agents under study. A second stimulator was coupled to the first through an R.C. delay circuit to provide a test shock after each tenth driving stimulus. Both stimuli were applied through the same pair of electrodes. The interval between the last driving stimulus and the test shock was varied by steps of 5 to 10 msec and the intervals between the corresponding ventricular responses were recorded. The functional refractory period of the atrioventricular transmission system was estimated as the minimal interval between two ventricular responses both propagated from the atrium.

Drugs. The drugs used were propranolol (kindly supplied by I.C.I. Pharmaceutical Division), adrenaline (Parke-Davis) and (±)-isoprenaline hydrochloride (Aludrine, Boehringer). Adrenaline and isoprenaline were administered into the superior vena cava by continuous infusion; propranolol was injected in single doses into a femoral vein.

RESULTS

Activity of the atrioventricular node

After destruction of the sinoatrial node the atrioventricular node does not, as a rule, assume a stable and fixed level of activity. Its spontaneous frequency is quite variable. In most of the experiments the rate varied between 60 and 120 per min, and in others it was so slow that any further depression after the administration of propranolol resulted in deterioration of the preparation and the experiment had to be abandoned. Low frequency was not the only disturbing factor in these experiments. Nodal impulses may sometimes originate from different sites, leading to variation in the configuration of the electrograms and often to beat-by-beat variations in the temporal relationship between atrial and ventricular activation. The results described below were obtained in preparations in which the following conditions were maintained: (1) the spontaneous nodal frequency was greater than 60 but less than 135 beats/min; (2) there was a single pacemaker, or only slight variation, particularly when the frequency was low. In fact, the sixteen experiments used to study automatism included only two in which slight variations of the pacemaker were observed. In the others, the pacemaker site appeared to be constant; and (3) the atrial response occurred at the same time or after the ventricular response (lower nodal pacemaker), or preceded the ventricular activation by less than 50 msec (upper nodal pacemaker).

Alterations of atrioventricular nodal activity in response to catechol amines

It is well known that adrenaline and isoprenaline augment the automatism of the atrioventricular node. With the doses we have used, 1 to 4 μg/kg/min of adrenaline and 0.1 to 0.4 μg/kg/min of isoprenaline, this effect is expressed not only as an increase in the frequency of the initially active pacemaker focus, but also by the appearance of new foci, recognized by variations in the temporal relationships between atrial and ventricular activity.

The nodal activity aroused by catechol amines is capricious. Frequently, pacemaker activity migrates successively from one region of the node to another. In some experiments the length of the atrioventricular interval and the configuration of the P-wave in lead II suggested that activity was initiated in the atrial muscle. Sometimes episodes of atrial fibrillation, or trains of atrial or ventricular extrasystoles, were recorded. Fig. 1 illustrates some of these reactions. In (*a*) may be seen the normal rhythm before, and in (*b*), the atrioventricular nodal rhythm after destruction of the sinoatrial node. In (*c*), recorded after administration of isoprenaline, the nodal rhythm is accelerated without apparent displacement of the pacemaker focus. In contrast, (*d*) and (*e*) from other experiments each show migration of the pacemaker site under the influence of isoprenaline. In (*d*) the shift was gradual; the temporal relationship between the atrial

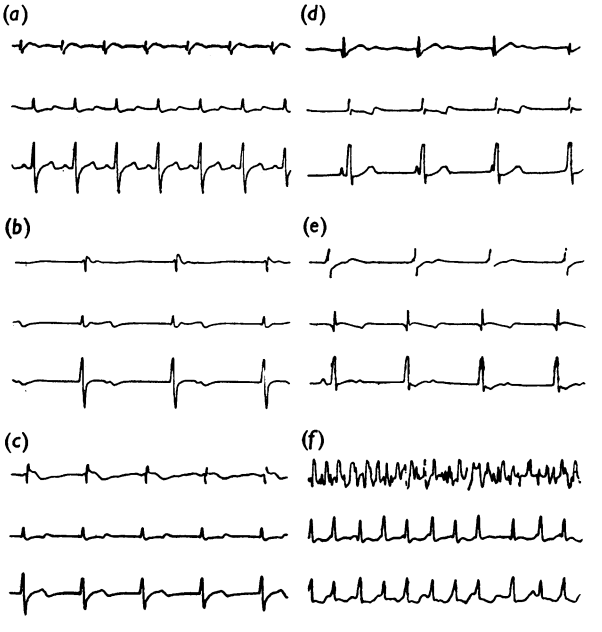

Fig. 1. Records of the activity of the atrioventricular node and representative responses of isoprenaline. In all parts of the figure, the upper trace corresponds to the auricular electrogram, the middle trace to the ventricular electrogram, and the lower to a lead II electrocardiogram, (*a*), (*b*) and (*c*) pertain to the same experiment. (*a*) Sinoatrial nodal rhythm; (*b*) atrioventricular nodal rhythm after destruction of the sinoatrial node; (*c*) acceleration of the nodal frequency during infusion of isoprenaline, 0.2 μg/kg/min; (*d*) and (*e*) migration of the atrioventricular nodal pacemaker under the influence of isoprenaline; (*f*) episode of atrial fibrillation induced by isoprenaline.

and ventricular electrograms and the P-R interval of the electrocardiogram suggests that the site of impulse formation moved downward from the upper to the middle level of the node. In (*e*) the pacemaker shifted abruptly from upper to lower levels in the course of successive impulses; in the first, atrial discharge precedes ventricular activation; in the others the ventricular complexes precede the atrial. In (*f*) the three tracings are consistent with a diagnosis of atrial fibrillation.

Action of propranolol on atrioventricular nodal automatism

When, after destruction of the sinoatrial node, the atrioventricular node assumes the pacemaker role, its automatic activity is diminished by propranolol. The doses used in the present study (from 0.003 to 0.2 mg/kg) depressed the spontaneous frequency by 10 to 40%. This limited degree of activity, and the great variation in the magnitude of the response to the same dose in different experiments, did not permit construction of a significantly valid dose/response relationship, at least within the dosage range used.

Action of propranolol on the chronotropic response of the atrioventricular node to adrenaline and isoprenaline

As with the sinoatrial node, propranolol can reduce or abolish the positive chronotropic responses of the atrioventricular node to adrenaline or isoprenaline. The antagonism appears to be of a competitive type, with a linear relation between dose and effect.

Fig. 2 summarizes the results. In (*a*) the chronotropic response of the atrioventricular node to adrenaline is expressed as a function of three dose levels, 1, 2 and 4 μg/kg/min for 5 min, before and after exposure to four different doses of propranolol. After the control responses to the three doses of adrenaline had been recorded, and after the spontaneous frequency had returned to the original level, the injection of propranolol further reduced the frequency. When, after 2 to 3 min, this had become stable infusions of adrenaline were repeated at the same three dose levels. Fig. 2 (*c*) expresses the relation between dose of propranolol and the corresponding percentage of block of the chronotropic response to adrenaline. (*b*) shows the antagonism of the effects of isoprenaline by propranolol. The three doses of isoprenaline were one-tenth of the corresponding doses of adrenaline. The only appreciable difference between the two sets of results is that the degree of antagonism is somewhat greater for isoprenaline, especially at the dose of 200 μg/kg of propranolol which caused nearly complete suppression of the effect of the highest dose of isoprenaline. (*d*) displays the regression line obtained from the isoprenaline results in the same way that (*c*) was constructed from the adrenaline results. The line is somewhat steeper, and the individual points are more dispersed. This is perhaps because isoprenaline in the doses used generally caused greater enhancement of automatism in various sites within the atrioventricular node, that is a "wandering" pacemaker.

The percentage of block with a given dose of propranolol was less at high doses of adrenaline and isoprenaline than at lower doses. Thus there was a significant negative correlation between the dose of adrenaline or of isoprenaline and the degree of blockade ($r = -0.724$, $P<0.001$; and $r = -0.723$, $P<0.001$; respectively).

The time required for the estimates of antagonism in each of the experiments with a specific dose of propranolol was approximately 20 min. On repetition of the same doses of adrenaline or isoprenaline 30 min later (that is 50 min after administration

of propranolol) some degree of block persisted, in rough proportion to the amount administered. At 50 min the degree of residual block was greatest for the highest dose of propranolol. We cannot define precisely the "half-life" of the response to the

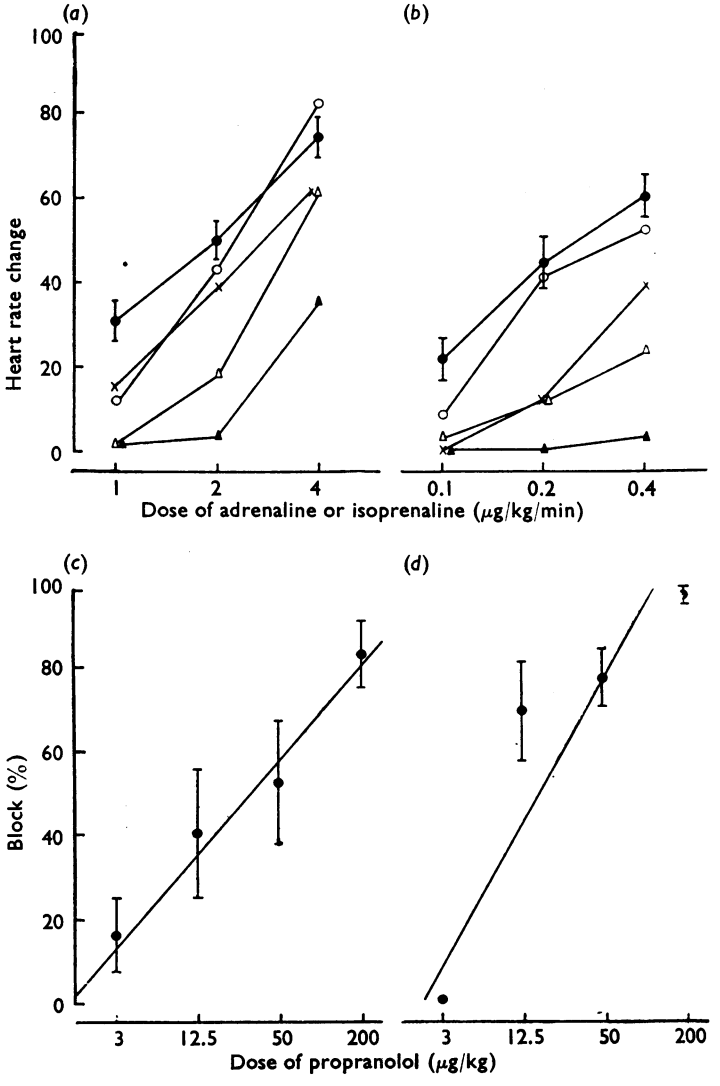

Fig. 2. Action of propranolol (single injection over approximately 1 min) on the positive chronotropic response of the atrioventricular node to adrenaline and isoprenaline. (*a*) Adrenaline: filled circles, mean values of control observations in eight experiments; empty circles, after propranolol, 3 μg/kg; crosses, 12.5 μg/kg; empty triangles, 50 μg/kg; filled triangles, 200 μg/kg. (*b*) Isoprenaline: symbols as in (*a*). (*c*) and (*d*) Regression line showing the relation between the dose of propranolol and block of the chronotropic response of the atrioventricular node to adrenaline (*c*) and to isoprenaline (*d*); points are means of six determinations, using the doses of (*a*) and (*b*), and the line represents the dose/effect relationship obtained by the method of least squares. Vertical lines indicate standard errors.

blocking agent, because the experimental preparation itself has a limited life. The acquisition of reliable dose/response curves 50 min after injection of the blocking agent was impeded by the progressive deterioration which occurred in some of the preparations.

Block of the action of adrenaline and isoprenaline on the refractory period of the atrioventricular transmission system

Propranolol can also block the abbreviation of the atrioventricular refractory period produced by adrenaline and isoprenaline. This action of propranolol was demonstrated in experiments in which all of the determinations of the refractory period (see Methods) were made at a constant driving frequency slightly higher than the spontaneous frequency resulting from catechol amine administration.

After obtaining control responses with adrenaline or isoprenaline, propranolol was injected and the doses of catechol amine were repeated. Fig. 3,*a* shows the shortening of the atrioventricular refractory period produced by an infusion of adrenaline, 1 μg/kg/min for 7 min. The refractory period was diminished from the control level of 236 msec to 206 msec by adrenaline. Administration of propranolol, 0.1 mg/kg, caused a slight increase which may be attributed to blockade of the action of the circulating endogenous catechol amines. On repeating the infusion of adrenaline almost total block was observed.

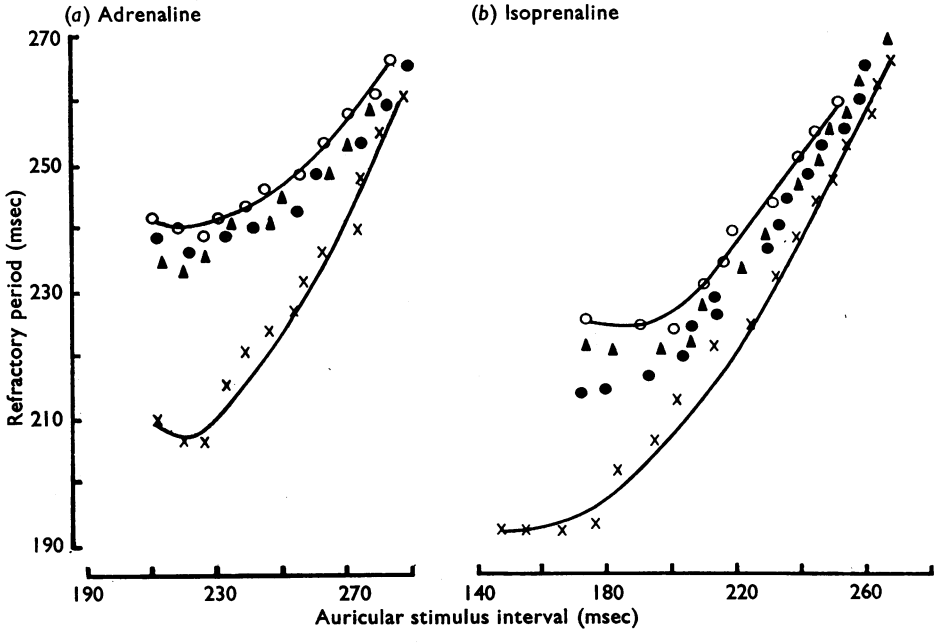

Fig. 3. Action of propranolol on the effects of adrenaline and isoprenaline on the functional refractory period of the atrioventricular transmission system. (*a*) Adrenaline; (*b*) isoprenaline. Filled circles, control curves; crosses, during infusion of adrenaline (1 μg/kg/min) or isoprenaline (0.1 μg/kg/min); empty circles, after propranolol (0.1 mg/kg); triangles, during infusion of the same doses of adrenaline or isoprenaline after propranolol. Ordinate, refractory period determined from the minimum interval between two ventricular electrographic responses both propagated from the atrium; abscissa, time interval between the corresponding pairs of shocks applied to the right auricular appendage.

The curve showing the influence of adrenaline after propranolol could be practically superimposed upon that for propranolol alone. Fig. 3,*b* illustrates similar results with isoprenaline in the approximately equieffective dose of 0.1 μg/kg/min.

Propranolol, 0.1 mg/kg, completely blocked the action of adrenaline (1 μg/kg/min) and that of isoprenaline (0.1 μg/kg/min). Smaller doses of propranolol (0.003 to 0.05 mg/kg) produced partial block. Effects comparable with those shown in Fig. 3 were demonstrated in three experiments with each of the catechol amines.

Augmentation of the pressor effect of adrenaline and abolition of the hypotensive effect of isoprenaline by propranolol

From the continuous record of arterial pressure which was made routinely in all experiments (all animals were vagotomized), it was noted that the pressor action of adrenaline, in doses of 1, 2 and 4 μg/kg/min, was always greater after injection of propranolol. Measurement of the control responses in eight experiments and of the response after propranolol in two experiments at each of the four dose levels yielded the results summarized in Fig. 4,*a*. The doses of propranolol were the same as in the experiments of Fig. 2. Potentiation of the pressor effect increased with increasing doses of propranolol except for the highest dose (0.2 mg/kg). It is possible that at this dose level the local anaesthetic action of propranolol becomes apparent (Morales-Aguilera & Vaughan Williams, 1965). These results are in harmony with those of Moran & Perkins (1958) with dichloroisoprenaline, and of Donald, Kvale & Shepherd (1964) with

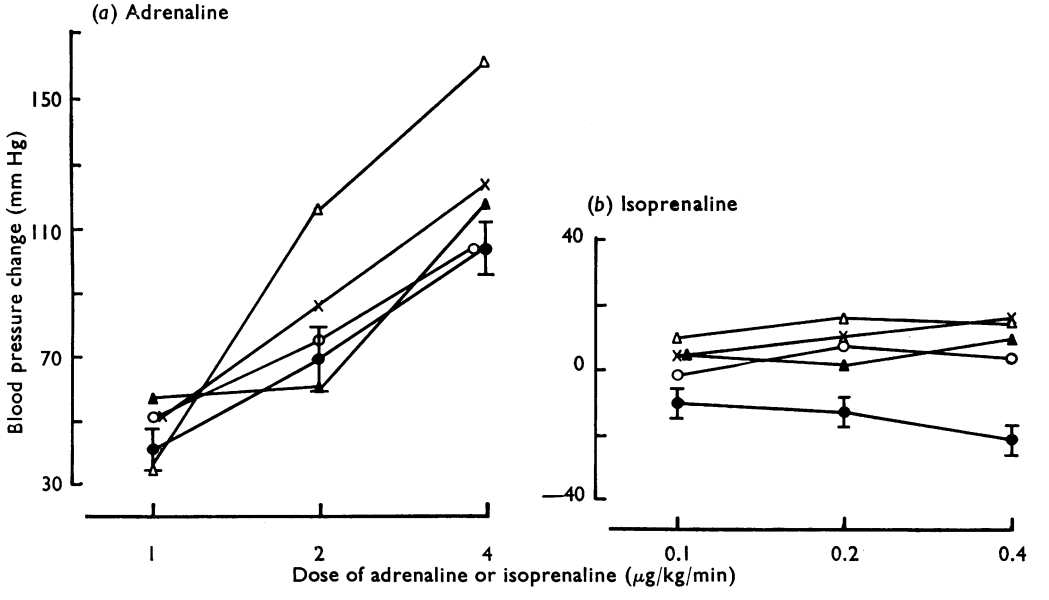

Fig. 4. Action of propranolol on the pressor effect of adrenaline and the depressor effect of isoprenaline. (*a*) Adrenaline; (*b*) isoprenaline. Filled circles, control; empty circles, after propranolol, 0.003 mg/kg; crosses, 0.0125 mg/kg; empty triangles, 0.05 mg/kg; and filled triangles, 0.2 mg/kg. Vertical lines indicate standard errors. Ordinate, change in blood pressure; abscissa, doses of adrenaline and isoprenaline in μg/kg/min.

pronethalol. In all three instances, augmentation of the pressor response to adrenaline is probably due to block of its vasodilator effects resulting in a predominance of the vasoconstrictor action.

Fig. 4,*b* shows the action of propranolol on the depressor responses to isoprenaline. The four doses of propranolol block the reduction of arterial resistance produced by isoprenaline, but the highest dose appears to be somewhat less effective than smaller doses, again suggesting the intervention of the secondary local anaesthetic action.

DISCUSSION

Analysis of our results demonstrates a sensitivity of the atrioventricular node to propranolol comparable with that exhibited by the sinoatrial node. Propranolol decreases the spontaneous atrioventricular nodal frequency and blocks the positive chronotropic action of adrenaline and isoprenaline, actions which are consonant with the known β-receptor blocking action of the compound. To these effects may be added, also as manifestations of β-receptor blockade, the antagonism of the actions of adrenaline and isoprenaline on the atrioventricular refractory period, the augmentation of the pressor action of adrenaline, and the suppression of the depressor action of isoprenaline.

The discovery that pronethalol has a potent local anaesthetic action (Gill & Vaughan Williams, 1964) and the recent study of Morales-Aguilera & Vaughan Williams (1965) according to which propranolol also shares this action, forces us to consider whether our results are due exclusively to adrenergic blockade or in part to a local anaesthetic action.

We believe that all of our results may be explained on the basis of adrenergic blockade which, in accordance with current concepts, we would include in the category of competitive antagonism. We base this assertion on the following arguments. (1) The range of doses used in the present study is much lower than the concentrations used in the experiments of Morales-Aguilera & Vaughan Williams (1965); our highest dose was 0.2 mg/kg whereas the effective local anaesthetic concentrations used by these authors were above 2 mg/l. However, as mentioned in the description of the action of propranolol on the arterial pressure responses to adrenaline and isoprenaline, it is possible that some action other than pure adrenergic blockade may begin to evidence itself at a dose as low as 0.2 mg/kg. (2) The depression of spontaneous atrioventricular nodal frequency and the increase of the atrioventricular refractory period caused by propranolol are probably due to antagonism of the tonic action of the cardiac sympathetic nerves and to block of the effects of circulating endogenous catechol amines. A direct action of the blocking agent on pacemaker discharge appears to be excluded by the experiments of Donald *et al.* (1964), who showed that pronethalol fails to cause bradycardia in animals subjected to chronic cardiac denervation. (3) Augmentation of the pressor action of adrenaline, as well as suppression of the hypotensive action of isoprenaline, are both in accord with a blockade of sympathetic β-receptors in the vessels of skeletal muscle. (4) Statistical analysis of our results with respect to the antagonism between propranolol and the catechol amines on the chronotropic responses of the atrioventricular node, with the correlation coefficients and P values mentioned, indicates a competitive blockade.

SUMMARY

1. In the anaesthetized dog, subjected to bilateral vagotomy and exclusion of the sinoatrial node, propranolol in doses of 0.003 to 0.2 mg/kg depresses the automatism of the atrioventricular node by 10 to 40%, with considerable variability in the response.

2. Propranolol antagonizes the positive chronotropic action of adrenaline and isoprenaline on the atrioventricular node in a precise dose/effect relationship. The antagonism has the characteristics of a competitive inhibition.

3. Similarly, propranolol antagonizes the abbreviation of the refractory period of the atrioventricular transmission system caused by adrenaline and isoprenaline.

4. Propranolol augments the pressor action of adrenaline, probably by blocking its vasodilator effects with consequent predominance of its constrictor action. Propranolol also blocks the hypotensive action of isoprenaline.

5. All of these effects of propranolol are the consequence of β-receptor blockade.

REFERENCES

BLACK, J. W., CROWTHER, A. F., SHANKS, R. G., SMITH, L. H. & DORNHORST, A. C. (1964). A new adrenergic beta-receptor antagonist. *Lancet*, **i**, 1080–1081.

DONALD, D. E., KVALE, J. & SHEPHERD, J. T. (1964). The effect of a beta-receptor antagonist on the cardiovascular system of the dog. *J. Pharmacol. exp. Ther.*, **143**, 344–349.

GILL, E. W. & VAUGHAN WILLIAMS, E. M. (1964). Local anaesthetic activity of the β-receptor antagonist pronethalol. *Nature (Lond.)*, **201**, 199.

GLOVER, W. E. & HUTCHISON, K. J. (1965). The effect of a beta-receptor antagonist (propanolol) on the cardiovascular response to intravenous infusion of noradrenaline in man. *J. Physiol. (Lond.)*, **177** 59–60*P*.

KRAYER, O., ARORA, R. B. & MEILMAN, E. (1955). Studies on veratrum alkaloids. XXI. The action of veratramine upon impulse generation in the dog heart. *J. Pharmacol. exp. Ther.*, **113**, 446–459.

KRAYER, O., MANDOKI, J. J. & MENDEZ, C. (1951). Studies on veratrum alkaloids. XVI. The action of epinephrine and of veratramine on the functional refractory period of the auriculo-ventricular transmission in the heart–lung preparation of the dog. *J. Pharmacol. exp. Ther.*, **103**, 412–419.

MORALES-AGUILERA, A. & VAUGHAN WILLIAMS, E. M. (1965). The effect on cardiac muscle of β-receptor antagonist in relation to their activity as local anaesthetics. *Brit. J. Pharmacol.*, **24**, 332–338.

MORAN, N. C. & PERKINS, M. E. (1958). Adrenergic blockade of the mammalian heart by a dichlor analogue of isoproterenol. *J. Pharmacol. exp. Ther.*, **124**, 223–237.

SHANKS, R. G. (1965). The pharmacology a new β-adrenergic receptor antagonist. *Pharmacologist*, **6**, 166.