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Mechanisms underlying the antiarrhythmic properties of β-adrenoceptor blockade against ischaemia-induced arrhythmias in acutely prepared rats

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1 The mechanism underlying the limited antiarrhythmic effects of β-adrenoceptor blocking agents against occlusion-induced arrhythmias in acutely prepared, pentobarbitone-anaesthetized rats has been investigated.

2 Atenolol, ICI 111,581 and propranolol were given at low, medium and high doses calculated to shift dose-response curves to exogenous agonists by factors of 10–30, 100–300 and 1000–3000, respectively.

3 Arrhythmias, blood pressure, heart rate, ECG changes and serum K^+ were measured.

4 Antiarrhythmic activity was seen with β-blocker treatment. This was minimal with atenolol (0.1, 1 and 10 mg kg^{-1}) and only statistically significant with the highest dose of ICI 111,581 (5 mg kg^{-1}), and propranolol (10 mg kg^{-1}).

5 Treatment with β-adrenoceptor blockers elevated serum potassium concentrations, as compared with saline controls, especially when measured at 30 min post-occlusion.

6 Only ICI 111,581 (5 mg kg^{-1}) and propranolol (1 and 10 mg kg^{-1}) prolonged P–R interval.

7 In order to evaluate possible mechanisms of antiarrhythmic action, attempts were made to correlate antiarrhythmic activity with β-blockade, serum potassium concentrations, and/or with changes in the P–R interval of the ECG.

8 Reductions in arrhythmias did not correlate well with presumed β-blockade. Better correlation was obtained with elevations of serum potassium concentration, and with prolongation of P–R interval (a presumed Class I antiarrhythmic action).

9 These results suggested that antiarrhythmic effects of adrenoceptor blocking agents in acutely-prepared anaesthetized rats, subjected to occlusion of a coronary artery, are unrelated to cardiac β-blockade. The limited antiarrhythmic effects which were observed could be attributed to elevations in serum potassium concentration (due to peripheral β-blockade) and/or possible Class I antiarrhythmic actions.

Introduction

The role the sympathetic nervous system plays in determining the duration and severity of occlusion-induced arrhythmias in the rat has been investigated in several studies. In our laboratory the problem has been examined by use of various approaches: CNS ablation (Curtis *et al.*, 1985b), tetrodotoxin-induced autonomic nervous system blockade (Abraham *et al.*, 1988) as well as β-adrenoceptor blockade in acutely (Au *et al.*, 1983) or chronically prepared animals, both conscious (Botting *et al.*, 1983) and anaesthetized (Au *et al.*, 1983). Findings in these studies for the most part refuted the hypothesis that the sympathetic nervous system plays anything more than a minor arrhythmogenic role during myocardial ischaemia in the rat. Beta-blockade with propranolol at doses sufficient to produce full β-blockade had only a minor effect on the severity and duration of arrhythmias (Au *et al.*, 1983; Botting *et al.*, 1983). However, other studies in conscious (Siegmund *et al.*, 1979; Szekeres, 1979), and acutely-prepared anaesthetized rats (Campbell & Parrat, 1983) have demonstrated antiarrhythmic activity with β-receptor antagonists such as propranolol.

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The strongest evidence for the antiarrhythmic activity of β-receptor blocking agents originates in acutely-prepared anaesthetized animals. We have previously suggested (Curtis *et al.*, 1985a; Curtis *et al.*, 1986) that antiarrhythmic effects of β-blockers in such preparations are secondary to a β-blockade-dependent elevation of serum potassium levels. Elevated levels of serum K^+ have been documented in acute surgery, and elevation of serum K^+ has been shown to be antiarrhythmic, both clinically and experimentally (Nordrehaug & von der Lippe, 1983; Solomon, 1984; Curtis *et al.*, 1986). Catecholamines depress serum K^+ via a β_2-adrenoceptor mediated action (Brown *et al.*, 1983). This would suggest that, if β-antagonists are antiarrhythmic in acutely prepared anaesthetized rats, it may be because of their ability to antagonize the catecholamine-mediated supression of surgically-induced elevations in serum K^+. In addition, pentobarbitone anaesthesia probably elevates sympathetic tone and may also impair the ability of the body to regulate serum potassium. If the above is correct, it would explain the apparent contradiction between results obtained with β-blockers in acutely-prepared anaesthetized rats and those obtained in conscious animals. In the present study we assessed the anti-arrhythmic properties of atenolol (a 'selective' β_1-adrenoceptor blocker), ICI 111,581 (β_2) and propranolol (non-selective) in acutely-prepared pentobarbitone-anaesthetized rats. Antagonists were administered at low, medium and high doses designed to ensure adequate β-blockade.

Methods

Male Sprague-Dawley rats (250–400 g) were used throughout. All experiments were performed in pentobarbitone anaesthetized (60 mg kg^{-1}, i.p.) and artificially ventilated rats (Palmer Small Animal Ventilator) at a stroke volume and rate which keeps blood gases in the normal range.

Groups studied, doses and route of administration

The occlusion study was performed in 12 groups of animals in 3 stages with 3 saline-treated groups. Each group contained 9 rats. The three blockers were each administered at three dose levels expected to shift dose-response curves for dobutamine, salbutamol or isoprenaline, as appropriate to the antagonist, by factors of 10–30, 100–300 and 1000–3000. A total of 1 ml of the appropriate concentration of drug was administered to the animal over a 5 min interval and then the rate of infusion was slowed to 0.5 ml h^{-1} so as to maintain a constant level of blockade.

Preliminary experiments were conducted to ascertain the appropriate dose (bolus and infusion) regimens required to achieve the above blockade. These preliminary studies were performed in rats anaesthetised with pentobarbitone (60 mg kg^{-1}, i.p.) and with a cannulated femoral vein and artery. Heart rate and blood pressure responses were obtained for salbutamol, isoprenaline and dobutamine before and after administration of the β-blocker under test. After these initial dose-range studies the minimal doses chosen were 0.1 mg kg^{-1} propranolol, 0.1 mg kg^{-1} atenolol and 0.05 mg kg^{-1} ICI 111,581. The shifts in partial dose-response (blood pressure and heart rate) curves to the above three agonists were used to estimate dose-ratios associated with the low doses. After this measurement, the dose of antagonist under study was increased by a factor of 10. Dose-ratios for falls in blood pressure to salbutamol and isoprenaline were 20 for the low dose of ICI 111,581 and propranolol. Ratios of approximately 100–200 were found with 1 mg kg^{-1} propranolol and 0.5 mg kg^{-1} ICI 111,581. When measured, ratios increased approximately a further 10 times with the highest doses of these two antagonists. Similar ratios were found for heart rate responses with propranolol, whereas no shift in heart rate occurred with ICI 111,581 until 5 mg kg^{-1} was given.

With isoprenaline and dobutamine as agonists, atenolol (0.1 mg kg^{-1}) gave a dose-ratio of 30 for heart rate while the ratio for blood pressure effects was less than 10. At 1 mg kg^{-1}, atenolol gave a dose-ratio of greater than 200 for heart rate and less than 30 for blood pressure. These data were considered in keeping with the limited selectivity of atenolol for the β_1-adrenoceptor and the greater selectivity of ICI 111,581 as a β_2-adrenoceptor blocker.

Experiments were conducted according to the Lambeth Conventions (Walker *et al.*, 1988). Animals were allocated to each of the 12 groups in a double-blind and randomized manner. Rats were excluded from the study if they met exclusion criteria, which included a fall in mean blood pressure below 60 mmHg before drug administration, and/or an occluded zone (ischaemic ventricular mass) below 25%, or above 45%, of the total ventricular mass; eleven rats were so excluded.

Coronary occlusion

Antiarrhythmic actions against occlusion-induced arrhythmias were studied in pentobarbitone-anaesthetized and acutely-prepared rats implanted with a left anterior descending coronary artery (LAD) occluder, ECG electrodes (approximate lead V_3) and arterial and venous cannulae. The methods for preparation of rats, as well as the technique of occlusion, were according to those described by

Clarke *et al.* (1980). Animals were allowed 30 min to stabilize after completion of surgery and then drug administration was initiated. Animals were occluded by tightening the implanted ligature at time 0, i.e., 10 min after beginning drug administration. The variables measured were blood pressure (BP), heart rate (HR) and ECG changes (V_3 chest lead, Johnston *et al.*, 1983). Body temperature was measured by a rectal probe (YSI model 73A) and maintained at 37.0 ± 0.5°C by means of a heating lamp. Arrhythmia scores (AS) were calculated according to the occurrence of ventricular premature beats (VPB) and the number and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) episodes diagnosed according to the Lambeth Conventions (Walker *et al.*, 1988). At the end of experiment, i.e., 30 min after LAD occlusion, rats were killed and the occluded zone (OZ = zone-at-risk) measured (Johnston *et al.*, 1981; 1983). This involved perfusing the heart by the Langendorff method with a Krebs-Henseleit solution containing cardiac green so that the under-perfused tissue was clearly demarcated.

Three blood samples were taken (−15 min = control; −1 min = drug; +30 min = post-occlusion) from the carotid artery cannula for measurement of serum K^+ concentration by potassium ion selective electrodes (Ionetics Potassium Analyzer, Ionetics, CA, U.S.A.). In view of the perturbing effects of concomitant tachyarrhythmias and difficulty of obtaining blood samples while monitoring for arrhythmias on ECG and blood pressure traces, the +30 min post-occlusion value was taken as being indicative of values during the period of peak arrhythmias (8–12 min post-occlusion). The validity of this assumption was tested in trial experiments when blood was taken during the peak period of arrhythmias in control and propranolol-treated animals and compared with values obtained at +30 min. For controls ($n = 7$) the K^+ values at the peak of arrhythmias were 97 ± 5% of those at +30 min, while for propranolol the mean was 95%. During these experiments it was consistently noted that serum potassium values were elevated after the termination of a tachyarrhythmia in which blood pressure fell for more than a few seconds. Electrically-induced tachyarrhythmias similarly elevated serum potassium. Thus we decided to use the +30 min values as being indicative of those expected at the peak period for arrhythmias.

Drugs

Atenolol and (±)-propranolol HCl were purchased from Sigma Chemical Company St. Louis, Mo U.S.A.; ICI 111,581 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol-HCl) was a gift from the ICI Company, Macclesfield, UK.

Data analysis

Statistical analysis was performed by ANOVA and statistical significance determined by Duncan's multiple range test for means using UBC computer packages (Gregg & Osterlin, 1977).

Results

All groups had the same mean occluded zone size (range 32.3 ± 1.9 to 38.1 ± 1.4 $\bar{x}$ ± s.e.mean) and therefore were presumably subjected to the same basic arrhythmic insult although the arrhythmogenicity of this insult may be modified by drugs or concomitant pathology.

Prior to the administration of saline, or a β-adrenoceptor blocker (−15 min values), all groups had similar mean heart rates with values ranging from 363 ± 11 ($\bar{x}$ ± s.e.mean) to 406 ± 9 beats min^{-1}. Saline treatment did not lower heart rate, whereas β-blocker treatment did, especially at the higher doses. The falls in heart rate with treatment were statistically significant in 5 out of 9 of the treatment groups just before occlusion (−1 min values) when comparisons were made between pre-drug and post-drug periods, but all doses produced falls in heart rate. Changes reached significance for 5.0 mg kg^{-1} ICI 111,581 (382 ± 9 to 362 ± 11 beats min^{-1}), but much larger statistically significant falls were seen with 0.1 mg kg^{-1} and 10 mg kg^{-1} atenolol and 1 and 10 mg kg^{-1} propranolol. The appropriate pre- to post-drug falls in heart rate with atenolol were 410 ± 18 to 330 ± 9 for 0.1 mg kg^{-1}, and 383 ± 11 to 293 ± 5 for 10 mg kg^{-1}. The corresponding figures for 1 and 10 mg kg^{-1} propranolol were 400 ± 15 to 365 ± 9, and 378 ± 14 to 280 ± 18, respectively.

All treatments tended to lower blood pressure when compared with their own control, unlike the case with saline where no significant falls in blood pressure occurred in saline-treated animals between the −15 min (pre-drug) and −1 min (post-drug) periods. Atenolol statistically significantly lowered blood pressure only at the 10 mg kg^{-1} dose (from 104 ± 5 to 69 ± 6 mmHg). With ICI 111,581 no statistically significant falls occurred while significant falls occurred with propranolol at 0.1 and 10 mg kg^{-1}. The above analyses involved pre-drug vs. post-drug comparisons. When post-drug values for drug-treated groups were compared with the same values for saline-treated controls, statistical significance was obtained with 10 mg kg^{-1} atenolol, 5 mg kg^{-1} ICI 111,581 and 0.1 and 10 mg kg^{-1} propranolol.

Treatment effects on serum potassium concentrations are shown in Figure 1. To illustrate fully the extent of such effects, values are expressed as changes

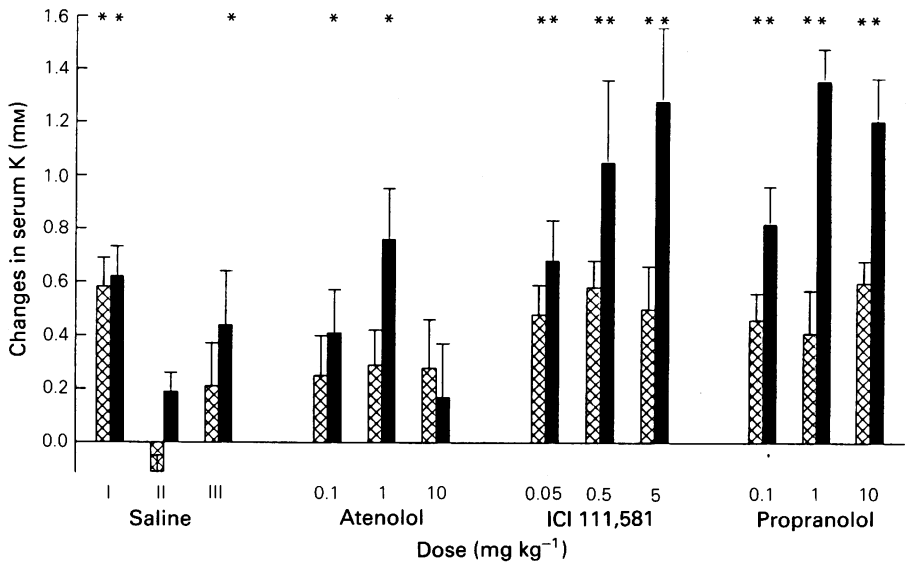

Figure 1 Effects of atenolol, ICI 111,581 and propranolol treatment on elevations in serum potassium concentrations in pentobarbitone anaesthetized rats. Drugs were administered as indicated in Methods and occlusion performed 10 min after beginning treatment. The values for the saline groups represents the control three groups. Results are presented as a change ($\bar{x}$ with s.e.mean shown by vertical bars of 9 rats per group) in serum potassium concentration from the initial pre-drug value, either 1 min before occlusion (hatched columns), or 30 min after occlusion (solid columns). $^*P < 0.05$ for significance of changes.

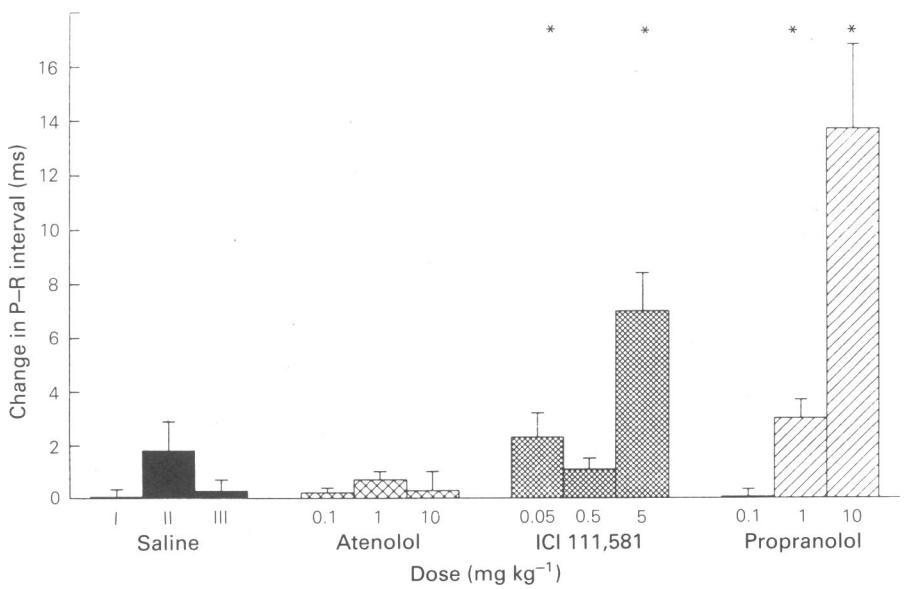

Figure 2 Effects of atenolol, ICI 111,581 and propranolol on P–R intervals in pentobarbitone anaesthetized rats. Drugs were administered as indicated in Methods. Each column is the mean with vertical bars showing s.e.mean; ($n = 9$) change in P–R interval 1 min before occlusion. $^*P < 0.05$ for difference.

from control. The control was the −15 min value which was obtained after surgery was complete, but just before starting treatment. Such control values varied between 3.4 ± 0.2 ($\bar{x} \pm$ s.e.mean) and 4.0 ± 0.2 mM. In all cases, with the exception of saline group II, there was an elevation at both −1 and +30 min and these elevations were generally statistically significant as a difference from their own control value. However, for saline groups I and III there were no statistically significant differences between the −1 and +30 values. On the other hand, in the β-blocker-treated groups, the elevation at +30 min was always statistically significant. In terms of the efficacy of the different β-blockers in raising serum potassium, both propranolol and ICI 111,581 may have been more efficacious than atenolol, especially prior to occlusion.

In terms of the ECG effects of β-blockade, the pattern of changes in acutely prepared animals were difficult to measure and analyse, compared with chronically prepared animals. As a result we only considered P–R intervals. Somewhat surprisingly, β-blockade (−15 min value versus −1 min value) did not cause statistically significant prolongation of the P–R interval, except in the case of the highest dose of ICI 111,581 and the two highest doses of propranolol (Figure 2). In the case of the latter drug, the prolongation appeared dose-related.

The effects of treatment on arrhythmias are summarized in Table 1. Statistically significant antiarrhythmic effects were only seen with the highest doses of ICI 111,581, and propranolol. With propranolol an increasing antiarrhythmic effect occurred with increasing doses. The above analysis was for VT and VF. The only statistically significant reduction in VPB occurred with the high dose of ICI 111,581. Since the arrhythmic history of an animal is adequately summarized by an arrhythmia score (Curtis & Walker, 1988) this is included in Table 1 as a summary.

In order to search for relationships between the various measured variables and arrhythmias, correlation analyses were performed. Figure 3 (a and b) shows two findings of significance. In Figure 3a the relationship between arrhythmia scores (as a percentage change from control values) and the +30 min serum potassium concentration (as $\log_e$ of elevation from control values) is shown. The linear correlation between the two variables was statistically significant as was the linear correlation between prolongation of P–R interval and the percentage reduction in arrhythmia score (Figure 3b).

Discussion

The three drugs in this study were chosen on the basis of their respective β-blocking profiles; atenolol for its partial β_1-adrenoceptor selectivity, ICI 111,581 for its β_2 selectivity, and propranolol for its lack of selectivity, as well as Class I antiarrhythmic activity. Atenolol has no reported Class I activity while if ICI 111,581 has, it has not been reported. The doses chosen were designed to span a full range

Table 1 Effects of atenolol, ICI 111,581 and propranolol on the occurrence of occlusion-induced arrhythmias in rats

Group	Dose ($mg\,kg^{-1}$)	Incidence (Number in groups having) VT	VF	VT and/or VF	log_{10} VPB	Arrhythmia score
Saline I		8	4	9	1.7 ± 0.2	4.1 ± 0.5
II		8	3	8	2.1 ± 0.1	3.4 ± 0.4
III		9	4	9	1.8 ± 0.2	3.7 ± 0.5
Atenolol	0.1	8	4	8	2.0 ± 0.1	3.6 ± 0.5
	1	8	4	8	1.8 ± 0.1	3.2 ± 0.5
	10	8	5	8	1.4 ± 0.3	3.3 ± 0.6
ICI 111,581	0.05	8	5	8	2.0 ± 0.2	3.7 ± 0.5
	0.5	9	4	9	1.8 ± 0.1	4.1 ± 0.4
	5	4	2	4	0.9 ± 0.3*	1.6 ± 0.7*
Propranolol	0.1	8	2	8	1.7 ± 0.1	3.3 ± 0.5
	1	6	1	6	1.5 ± 0.3	2.9 ± 0.6
	10	3*	2	3*	1.7 ± 0.2	1.1 ± 0.4*

Drugs were administered as a bolus followed by an i.v. infusion as described in Methods. Occlusion was performed 10 min after the beginning of treatment. Values are expressed as incidence (number of rats per group of 9 having VT, VF, VT and/or VF) or $\bar{x} \pm$ s.e.mean ($\log_{10}$ VPB) of 9 rats per group. Arrhythmia score (AS) is a normally distributed, linearly additive score which summarises an animal's arrhythmic history. *$P < 0.05$ vs. saline.

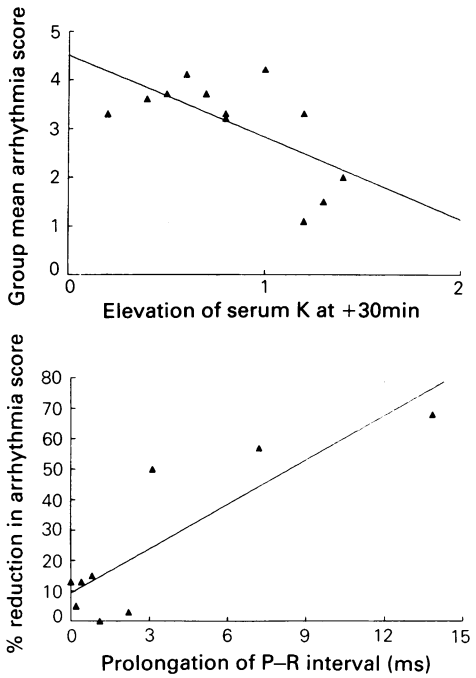

Figure 3 Correlation analyses between arrhythmia score as a percentage of control and elevated serum K^+ levels ($\log_e$ mM) (a); as well as the percentage reduction in arrhythmia score from control and P–R interval (b). In (a) the antiarrhythmic effect is shown as a decrease in arrhythmia scores with increasing elevations in serum potassium values (as $\log_e$ mM) compared with control values. The correlation coefficient r was 0.84 ($P < 0.001$). In (b) the decrease in arrhythmia scores from control values is plotted against the change from pre-drug values in P–R interval 1 min before occlusion. The correlation coefficient was 0.86.

of β-blockade. At the lowest doses, dose-ratios of 10–30 could be expected to reduce severely any normal activity of endogenous noradrenaline, or adrenaline, on β_1-, β_2 and/or β_1-plus β_2- adrenoceptors. The highest doses could be expected to block completely (99.9% blockade) even the highest level of endogenous noradrenaline likely to be found in ischaemic tissue.

With regard to their pharmacological actions, all three drugs lowered heart rate. This was so even with the selective β_2-antagonist. At the highest doses it can be presumed that the selectivity of both selective antagonists was minimal, especially with atenolol which has a limited selectivity (Tabrizchi, 1988). This is reflected in the fact that the highest dose of all three drugs produced similar elevations of serum potassium +30 min after occlusion. This potassium elevating action was presumably due to blockade of β_2-adrenoceptors the activation of which lowers serum potassium (Brown *et al.*, 1983). In this study it would not have been helpful to measure serum potassium concentrations at the peak time for the appearance of arrhythmias because of technical difficulties and confounding effects of recent arrhythmias. The decision was taken not to withdraw blood at this period for the major study. It appeared from the preliminary studies that +30 min values probably accurately reflect the levels at the time of peak arrhythmias assuming that no arrhythmias occurred to elevate values artificially. While making serum potassium determinations in many studies we have consistently found that short periods (s), even of relative ischaemia, elevate serum potassium. Thus, it is advisable to avoid sampling for serum potassium levels when blood pressure is liable to fall precipitously for varying periods as a result of the occurrence of arrhythmias.

In view of the possibility of Class I actions (blockade of sodium conductance) with high doses of propranolol, we attempted to measure Class I actions by monitoring the P–R interval of the ECG. This was done, despite the realization that β_1-adrenoceptor blockade could also be expected to prolong P–R. However, we have consistently found (Curtis *et al.*, 1985a,b; Curtis & Walker, 1986; Johnston *et al.*, 1983; Abraham *et al.*, 1988) that Class I antiarrhythmics are more effective than even calcium antagonists in prolonging the P–R interval of the rat. Interestingly, the presence of β-blockade, as judged by bradycardia, did not prolong the P–R interval, whereas high doses of propranolol and the highest dose of IC 111,581 did.

In view of the findings of bradycardia, elevated serum potassium, prolonged P–R interval, and limited antiarrhythmic effectiveness of β-blocker treatment, it was important to try and establish a causal relationships between these events. In the first place there was no clear correlation between the expected degree and efficacy of β_1-blockade (bradycardia) and antiarrhythmic effects. On the other hand, statistically significant reductions in arrhythmias correlated with prolongation of P–R interval (Figure 3b). Since large doses of ICI 111,581 and propranolol had to be given to cause P–R prolongation, we have identified prolongation as being indicative of sodium channel blockade. This is in agreement with previous observations that several β-blockers have actions on the Na^+ current in cardiac tissue (Morales-Aguilera & Vaughan Williams, 1965). Furthermore, atenolol, a drug with no reported sodium channel blocking actions, completely failed to prolong the P–R even at the highest doses. It is difficult to imagine that the highest dose of

atenolol was not producing complete cardiac β_1-adrenoceptor blockade.

The other correlation of interest was that between serum potassium and antiarrhythmic efficacy. According to Figure 3a, an e-fold rise in serum potassium could be expected to reduce arrhythmias by 40%. This can be compared with the value of 63% obtained by interpolation of data from previously reported studies (Figure 2, Curtis *et al.*, 1985a; Curtis *et al.*, 1986) and from an unpublished study in which 10 different groups of conscious rats, treated so as to produce hypo- or hyperkalaemia, were subjected to coronary occlusion. Extrapolation and calculation from the data in Figure 3a gave an arrhythmia score of 4.4 for a normal serum potassium which was the same score as that seen in untreated conscious rats (Johnston *et al.*, 1983). The lack of an absolutely unambiguous correlation in Figure 3a probably reflects the relative lack of precision in the measurement of arrhythmias in acutely-prepared anaesthetized rats.

From the above, we can tentatively draw the conclusion that any antiarrhythmic activity of β-blockers in acutely prepared pentobarbitone anaesthetized rats can be ascribed to mechanisms other than blockade of cardiac β_1-receptors. It is more likely that antiarrhythmic activity is due to a β-blockade-dependent elevation of serum potassium plus Class I antiarrhythmic actions. Parratt's group (Campbell & Parratt, 1983) have considered the latter possibility as being of secondary importance following an extensive series of investigations with a variety of β-blockers. Indeed, in conscious rats, enormous doses of propranolol (40 mg kg^{-1}, i.v. or more) have little antiarrhythmic activity (unpublished observations), despite being capable of inducing convulsions, presumably as a result of local anaesthetic actions in the brain.

It may be possible to reconcile the many apparent contradictions that are found in the literature concerning rats regarding the actions of β-blockers (see Curtis *et al.*, 1987) if the action of β-blockers on serum potassium are taken into consideration. In conscious rats serum potassium does not change with occlusion, and β-blockade or sympathectomy have no antiarrhythmic activity. Antiarrhythmic activity is only seen where recent surgical damage in the presence of presumed impairment of renovascular function results in a tendency for serum potassium to rise. Pentobarbitone anaesthesia in rats subjected to surgery is mostly associated with elevated heart rates and blood pressure (Au *et al.*, 1983) and, if this is taken as a sign of increased sympathetic activity, then the associated β-adrenoceptor activation can be expected to moderate any tendency for serum potassium to rise. Blockade of this β-adrenoceptor action can be expected to result in a further elevation of serum potassium. Finally, as speculation, it is interesting to wonder whether the ability of β-blockers to modulate serum potassium may be related to the ability of such drugs to reduce mortality in post-infarct patients.

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