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Cardiac actions in the dog of a new antagonist of adrenergic excitation which does not produce competitive blockade of adrenoceptors

R. CHARLIER

Department of Pharmacology, LABAZ Research Division, Avenue de Béjar 1, 1120 Brussels, Belgium

Summary

1. The cardiac actions of amiodarone, a benzofuran derivative used in the treatment of angina pectoris, have been compared with those of (±)-propranolol in anaesthetized dogs.

2. After three successive intravenous injections of propranolol, 0·5 mg/kg, had reduced the heart rate by 25%, a fourth dose had no further negative chronotropic action, but amiodarone, 10 mg/kg intravenously, at this point reduced the heart rate by 23%.

3. Amiodarone, 10 mg/kg intravenously, reduced, but did not abolish, cardiac responses to isoprenaline, 2 μg/kg intravenously. Subsequent successive injections of 10 mg/kg of amiodarone did not further block the responses to isoprenaline, but propranolol, 1 mg/kg intravenously, abolished them.

4. Amiodarone reduced cardiac chronotropic and inotropic responses to glucagon, which were not affected by propranolol.

5. Cardiac output was increased 5 min after amiodarone, 10 mg/kg intravenously, but at 10 min and thereafter it did not differ from control values. Propranolol, 1 mg/kg intravenously, reduced cardiac output by 17% at 5 min, and by 30% after 30 min.

6. From this and other evidence which is discussed, it is concluded that the cardiac actions of amiodarone are not produced by competitive blockade of β-adrenoceptors.

Introduction

Amiodarone, a benzofuran derivative [2-butyl-3-(3,5,diiodo-4-β-diethylaminoethoxybenzoyl)-benzofuran hydrochloride], which is a smooth muscle relaxant with a specificity for vascular muscle (Deltour, Binon, Tondeur, Goldenberg, Henaux, Sion, Deray & Charlier, 1962 ; Charlier, Deltour, Tondeur & Binon, 1962) has pharmacological properties which have been reviewed by Charlier, Deltour, Baudine & Chaillet (1968). Amiodarone increases coronary flow, but reduces both the work and oxygen consumption of the myocardium, and causes bradycardia, slight hypotension and a fall in overall vascular resistance (Charlier, Baudine, Chaillet & Deltour, 1967).

Amiodarone reduces the effects of sympathetic stimulation and the actions of injected catecholamines mediated by both α- and β-adrenoceptors (Charlier *et al.*,

1968), according to the classification of Ahlquist (1948). In this paper a comparison of the cardiac effects of amiodarone and (±)-propranolol has been undertaken, the results of which indicate that amiodarone does not achieve its cardiac effects in dogs by competitive blockade of β-adrenoceptors.

Methods

All experiments were performed on dogs (12–35 kg) of either sex anaesthetized with pentobarbitone sodium, 30 mg/kg intravenously, and intubated with a Rüsch tracheal cannula. The heart rate was monitored by a Sanborn cardio-tach preamplifier. Femoral blood pressure and left ventricular pressure were recorded with Sanborn 267B transducers and a 350-1100B carrier preamplifier (a catheter, Cournand No. 8, was passed into the ventricle via the left femoral artery). In some experiments a differential record (dp/dt) was also obtained (Sanborn 350-1500A preamplifier and 350-16 module). Cardiac output (Goodyer, Huvos, Eckhardt & Ostberg, 1959) was measured after heparinization (0·1 mg/kg intravenously) by injecting 3 ml saline at 24° C directly into the right atrium through a catheter (Cournand No. 8) passed from the right external jugular vein and recording changes of temperature with a thermistor probe (Yellow Springs Instrument Co.) at the tip of a Cournand No. 6 catheter located near the aortic valves and introduced via the left femoral artery. The output of the probe was fed into a Sanborn 350-1500A preamplifier, 350-15 thermal module and 130 cardiac output computer, and the calculated cardiac output was recorded on a Sanborn 356 multi-channel unit, on which the other outputs were also displayed.

Amiodarone was given intravenously in a 5% aqueous solution of the hydrochloride at a rate such that a dose of 10 mg/kg took 2 min to inject. Propranolol was administered in a 0·5% aqueous solution intravenously.

Drugs used. Amiodarone (used as hydrochloride; "Cordarone"; L 3428 LABAZ); pentobarbitone sodium (Nembutal, Abbott); heparin (Heparin, Boots); (±)-isoprenaline sulphate (Aleudrin, Boehringer); glucagon (Glucagon, Novo); atropine sulphate (P.B.V.); (±)-propranolol (synthesized in our Chemistry Department); (±)-adrenaline hydrochloride (P.C.B.).

In the case of salts all doses given refer to the salt.

Results

Effects on heart rate, mediated by β-adrenoceptors

Amiodarone itself induces a bradycardia which is not affected by atropine. 10 mg/kg of amiodarone, given intravenously, was previously reported to reduce the heart rate by 27% (Charlier *et al.*, 1968). To investigate the involvement of β-adrenoceptors, 0·5 mg/kg of propranolol was given intravenously to an anaesthetized dog 15 min after an intravenous dose of 1 mg/kg of atropine sulphate. It reduced the heart rate from 204 to 156 beats/min. A second and third injection of 0·5 mg/kg of propranolol again slightly decreased the heart rate, but a fourth dose had no additional effect (Fig. 1). Amiodarone, 10 mg/kg intravenously, however, given at this point, reduced the heart rate by a further 23%, from 147 to 114 beats/min. It is, therefore, unlikely that under the experimental conditions the bradycardia induced by amiodarone involved any effect on β-adrenoceptors.

In another experiment, 2 μg/kg of isoprenaline, given intravenously, increased the heart rate by 50%, but the same amount administered 10 min after 10 mg/kg of amiodarone increased the heart rate by only 17%. After a further 20 min (30 min after amiodarone) 2 μg/kg of isoprenaline increased the heart rate by 32%. A second injection of 10 mg/kg amiodarone was then given but caused a smaller reduction in the response to isoprenaline than was produced by the first dose, because the response to 2 μg/kg 10 min after this second dose of amiodarone was a 30% increase in the rate. It was evident, therefore, that amiodarone was not causing a progressive blockade of β-adrenoceptors. An injection of 1 mg/kg of propranolol was now given, as a result of which the response to 2 μg/kg of isoprenaline was completely abolished.

In other experiments, repeated doses of 10 mg/kg of amiodarone were given, interspersed with challenges by isoprenaline, up to a total of 70 mg/kg of amiodarone, but the response to isoprenaline after the last dose of amiodarone was no smaller than that after the first dose. Here again, it may be concluded that amiodarone is not a competitive blocker of β-adrenoceptors.

Finally, amiodarone, 10 mg/kg given intravenously, still caused bradycardia in a dog injected with 0·5 mg/kg of reserpine intraperitoneally 48 and 24 h before the experiment. It decreased the heart rate from 156 to 126 beats/min and reduced the positive chronotropic response to isoprenaline.

Effects on heart rate and contractions stimulated by glucagon

Glucagon increases heart rate, systolic left ventricular pressure and its rate of increase (dp/dt) in perfused isolated rat hearts (Laraia, Craig & Reddy, 1968) and

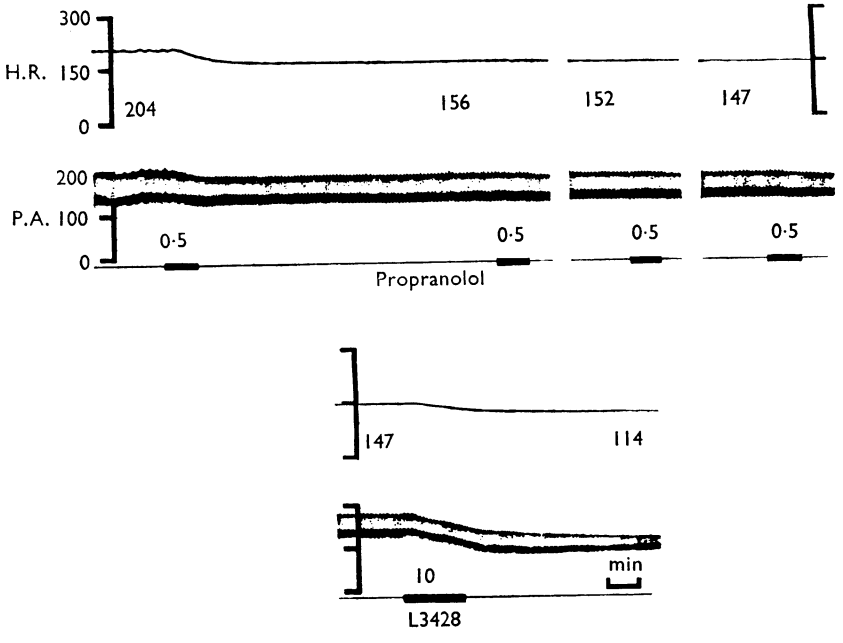

FIG. 1. Effects of (±)-propranolol and amiodarone (L 3428) on heart rate and blood pressure in an anaesthetized and atropinized dog. H.R., Heart rate (beats/min). P.A., Pressure in femoral artery (mmHg). The figures above the signal marks represent doses in mg/kg. For detail see text.

when given intravenously to anaesthetized animals (Lucchesi, 1968 ; Glick, Parmley, Wechsler & Sonnenblick, 1968). It also activates adenylcyclase (Levey & Epstein, 1969) in cat and human hearts. To test the involvement of effects mediated by glucagon in the action of amiodarone, glucagon 4, 5 and 25 μg/kg respectively was injected intravenously into anaesthetized dogs in three experiments, after records had been taken of heart rate and femoral blood pressure, and, in the third experiment, of the rate of change of left ventricular pressure also. The results are presented in Table 1, and it is evident that amiodarone reduced both the size and the duration of chronotropic responses to glucagon. Amiodarone also reduced the effect of glucagon on left ventricular pressure and its first derivative (Fig. 2). Propranolol, 1 mg/kg intravenously, on the other hand, did not modify the tachycardia induced by glucagon, in confirmation of the findings of Lucchesi (1968) and of Steiner, Wit & Damato (1969).

TABLE 1. *Effect of amiodarone, 10 mg/kg intravenously, on the chronotropic responses to intravenous glucagon in anaesthetized dogs*

Expt. No.	Dose of glucagon (μg/kg)	Maximum heart rate in response to glucagon		Time taken for 50% of effect to wear off (min)	
		Control	After amiodarone	Control	After amiodarone
1	4	+25%	+13%	25	15
2	5	+14%	+ 6%	20	12
3	25	+33%	+18%	38	20

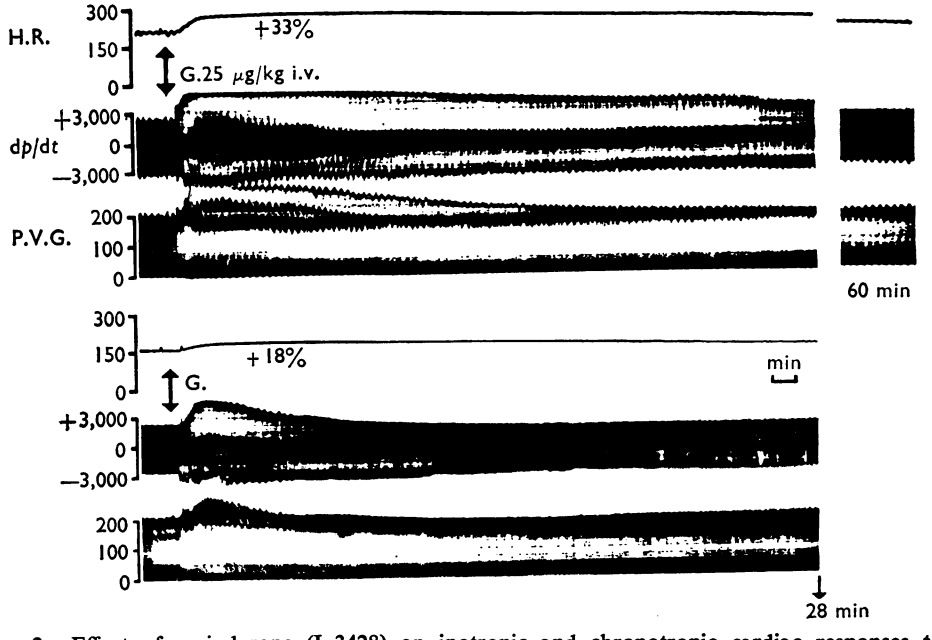

FIG. 2. Effect of amiodarone (L 3428) on inotropic and chronotropic cardiac responses to glucagon in an anaesthetized dog. H.R., Heart rate (beats/min). dp/dt, Rate of change of left ventricular pressure (mmHg/s). P.V.G.: Left ventricular pressure (mmHg). Upper trace: Control ; effect of 25 μg/kg of glucagon (G) given intravenously. Lower trace: Effect of same dose of glucagon after amiodarone, 10 mg/kg intravenously.

A check made on a dog which had not received any amiodarone showed that a second injection of glucagon produced quantitatively the same effect as that seen when it was given on the first occasion.

Effects on ventricular pressure and cardiac output

Adrenaline, 2 μg/kg intravenously, increased the rate of change of ventricular pressure (dp/dt) by 60%. Amiodarone, 10 mg/kg intravenously, reduced this increase by half, whereas propranolol, 0·5 mg/kg intravenously, abolished it altogether, confirming the observation of Benfey, Greeff & Heeg (1967).

In ten dogs five control observations of cardiac output were made at 5 min intervals. Six of the dogs then received 10 mg/kg of amiodarone intravenously and the other four 1 mg/kg of propranolol intravenously. Subsequent changes in cardiac output have been summarized in Fig. 3, each as a percentage of the mean of the five control observations. As can be seen amiodarone initially increased cardiac output, but after 10 min and thereafter the cardiac output was the same as that of controls. In contrast, after propranolol, cardiac output fell by 17% in the first 5 min, and at 30 and 35 min it had decreased by 30%. Sowton & Hamer (1966) also reported large falls in cardiac output after propranolol, and Moret, Boufas & Fournet (1969) recently confirmed that 5 and 10 mg/kg of amiodarone did not lower cardiac output in anaesthetized dogs.

Discussion

Amiodarone diminishes the effect of sympathetic stimulation or injected catecholamines in eliciting adrenergic responses mediated by both α- and β-adrenoceptors, since systemic hypertension induced by stimulation of the splanchnic nerve or by the intravenous injection of adrenaline or noradrenaline, as also the peripheral vasodilator action of isoprenaline and its positive chronotropic and inotropic actions

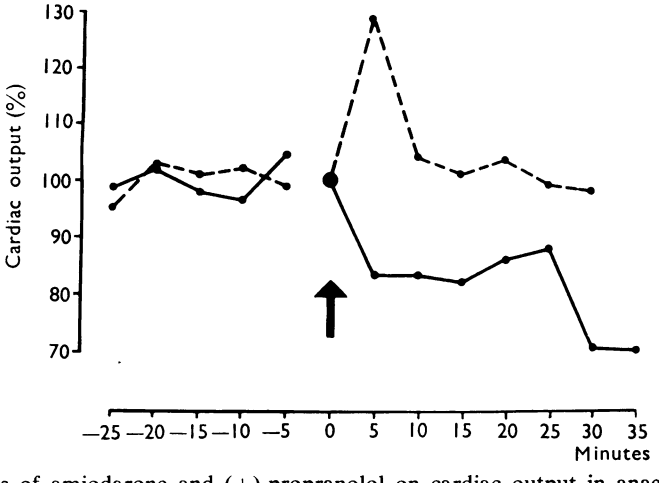

FIG. 3. Effects of amiodarone and (±)-propranolol on cardiac output in anaesthetized dogs. expressed as a percentage of the mean of five control readings. ●– –●, Mean results from six dogs given amiodarone, 10 mg/kg intravenously; ●——●, mean results from four dogs given (±)-propranolol, 1 mg/kg intravenously.

on the heart, are reduced by amiodarone (Charlier, Baudine & Chaillet, 1967; Charlier, Deltour & Baudine, 1967; Charlier, Baudine, Chaillet & Deltour, 1967).

Perhaps the most interesting feature of the pharmacological actions of amiodarone is that it reduces myocardial oxygen consumption and enhances coronary blood flow in the dog (Charlier *et al.*, 1968) without diminishing cardiac output. This is in contrast to the action of propranolol, which reduces cardiac output and coronary blood flow.

These actions of amiodarone are associated with biochemical changes in the myocardium. Amiodarone increases the ratio of ATP and creatine phosphate to ADP, creatine, and inorganic phosphate, and it only partially blocks the action of adrenaline to reduce this ratio and to reduce also the ratio of pyruvate to lactate, whereas the latter effects of adrenaline are completely abolished by propranolol (Broekhuysen, Deltour, Ghislain & Delbruyere, 1967; Broekhuysen, Laruel, Debrucq-Laruel & Deltour, 1967; Broekhuysen, Ghislain & Deltour, 1969).

Propranolol blocks adrenaline-induced lipolysis *in vivo* (serum free fatty acid) and *in vitro* (fat pad test) (Sailer, Sandhofer, Bolzano, Dienstl & Braunsteiner, 1967), but amiodarone does not affect these adrenergic phenomena, nor does amiodarone modify the increase in basal metabolic rate provoked by theophylline, whereas propranolol abolishes it (Cockburn, Hull & Walton, 1968).

The experiments reported here and in the paper by Vaughan Williams & Singh (1970) indicate that the anti-adrenergic actions of amiodarone are not mediated by competitive blockade of β-adrenoceptors. Furthermore pressor responses to adrenaline in anaesthetized dogs were reduced by 10 mg/kg of amiodarone, but were not further diminished when up to six additional injections of 10 mg/kg of amiodarone were given, indicating an absence of progressive α-adrenoceptor blockade. Conversely, propranolol did not affect the positive cardiac inotropic and chronotropic effects of glucagon, yet these were reduced by amiodarone. The antagonism by amiodarone to the cardiac actions of glucagon and catecholamines might both be explained by a reduced formation of cyclic 3′,5′-AMP. Broekhuysen (1969, unpublished) has found that 0·1 mM amiodarone inhibits the activation of adenylcyclase by adrenaline in rat heart homogenates, but studies of the inhibition of activation by glucagon are still incomplete.

Further evidence that amiodarone has effects independent of competitive adrenoceptor blockade is provided by the observation that, in an anaesthetized dog previously given atropine sulphate, 1 mg/kg, phentolamine, 4 mg/kg, and propranolol, 0·5 mg/kg, amiodarone, 10 mg/kg intravenously, still caused as great a bradycardia and hypotension as in an untreated animal (Charlier *et al.*, 1968). Amiodarone itself not only causes a small fall in mean blood pressure, but reduces the hypertensive response to intravenous adrenaline, whereas propranolol increases the pressor effect of adrenaline (Shanks, 1966). Further, amiodarone increases coronary flow, but propranolol reduces it (Charlier, 1968). Finally, peripheral vascular resistance is reduced by amiodarone, but increased by propranolol (McKenna, Corliss, Sialer, Zarnstorff, Crumpton & Rowe, 1966; Nakano & Kusakari, 1966; Shanks, 1966). From all the above evidence, it may be concluded that amiodarone does not act by competitive blockade of β-adrenoceptors.

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