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THE ADRENERGIC ANTAGONISM OF AMIODARONE*

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Abstract—Inhibition by amiodarone and propranolol of the chronotropic effect of isoproterenol on spontaneously beating rabbit right atria has been studied *in vitro*. Propranolol behaved like a typical competitive β_1-adrenoceptor antagonist with a pA_2 value of 8·33. Amiodarone acted as a non-competitive inhibitor with a pD'_2 value of about 4·17. Inhibition by amiodarone and phentolamine of the norepinephrine-induced contractions of isolated rat aortic strips has been studied likewise. Phentolamine was shown to inhibit the adrenergic α receptor competitively with a pA_2 value of 8·69. Amiodarone, though devoid of any appreciable effect on calcium permeability, acted again as a non-competitive inhibitor, with a pD'_2 value of about 4·06. The mechanism of action of amiodarone is discussed.

Amiodarone† has been shown to antagonize *in vivo* several cardiovascular effects of the catecholamines [1]. At the usual dose of 10 mg/kg i.v. in the anesthetized dog, it halves the hypertension induced by an injection of epinephrine or norepinephrine, or by electrical stimulation of the splanchnic nerve.

Catecholamine-induced vasoconstriction is also lowered, as measured by the blood flow in peripheral arteries. Isoproterenol-induced tachycardia and hypotension are similarly antagonized by about 50 per cent.

The α- and β-adrenergic antagonism seems not to be dose-dependent, and complete blockade of the adrenergic responses to a given dose of the agonist cannot be achieved. Moreover, amiodarone was shown to antagonize the chronotropic effects of glucagon in the same way [2].

These properties are strikingly different from either α- and β-adrenoceptor antagonists. Typical blocking agents, like phentolamine and propranolol, are specific and competitive inhibitors of only one receptor type: their action is dose-dependent and complete blockade is easily achieved.

Much work has been devoted to elucidate the nature of the adrenergic β receptors. The very first effect of their stimulation seems to be the activation of the membrane-bound enzyme adenylate cyclase [3], which triggers the intracellular increase of 3′,5′-cyclic adenosine monophosphate (cAMP) concentration. It has been shown *in vitro* on myocardial plasmic membrane preparations that amiodarone inhibits adenylate cyclase activation by catecholamines and glucagon [4]. It seemed therefore that the drug interferes with the early stages of hormonal stimulation, either at the receptor site or at the catalytic site of adenylate cyclase. However, amiodarone does not inhibit the unstimulated enzyme, and does not interfere with fluoride activation.‡ It would appear then, that amiodarone impinges on one of the receptor's sites located before adenylate cyclase.

Adrenergic stimulation of vascular smooth muscle, on the other hand, leads to an increase of intracellular free calcium concentration mediated by the α-adrenergic receptors [5]. An adrenergic antagonism could therefore arise either from drug–receptor interaction or from inhibition of calcium mobilization.

The aim of this work was to determine the nature of the adrenergic antagonism displayed by amiodarone. Three kinds of experiments have been performed. In order to elucidate the β-lytic mechanism of action, the effect on isoproterenol-induced frequency increase was studied on isolated rabbit right atria. For the α-lytic action, the effect on norepinephrine-induced contractions of isolated rat aortic strips was measured. The effect on K^+ depolarization-induced contractions of the same preparation was also studied in order to measure interaction with calcium transport.

MATERIAL AND METHODS

Isolated rabbit atria

Right atria from young rabbits (800–1700 g) of both sexes were quickly dissected and bathed in 25 ml modified Locke solution gassed with carbogen and maintained at 32°. The spontaneously beating preparation was connected to a Sanborn FTA 10–1 force transducer and the frequency was recorded from a Sanborn 350–3400 A cardiotach amplifier. A constant force of 1 g was applied to the organ.

After a stabilization period of 60 min, graded doses of isoproterenol were introduced, each increment being added when the preceding one had produced its highest effect. In this way, a control dose–response curve was established. The preparation was then washed until the spontaneous frequency was stabilized. The antagonist was introduced and the preparation left for 60 min before the dose–response curve was determined again.

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† 2-Butyl-3-(3,5-diiodo-4-β-diethylaminoethoxybenzoyl)-benzofuran hydrochloride. Cordarone®, Labaz.

‡ J. Broekhuysen and M. Ghislain, unpublished results.

It was ascertained on a number of preparations that the response obtained during the second period in the absence of antagonist was essentially equal to the first one. When a pD_2 of 8·76 ± 0·07 was obtained for isoproterenol during the first period, a value of 8·82 ± 0·08 was recorded during the second one (n = 14, P > 0·5 by *t*-test).

Isolated rat aortic strips

The aorta from male or female rats (weighing about 280 g) was taken from the left carotid to the diaphragm. It was immediately dissected, cut in a spiral and bathed in 25 ml modified Krebs–bicarbonate solution gassed with carbogen and maintained at 37°. A constant tension of 2 g was applied and maintained during the stabilization period. The preparation was connected to a Sanborn FTA 10-1 force transducer and the contractile responses were recorded.

(a) *Norepinephrine-induced contractions.* After a stabilization period of 90 min, a dose–response curve was obtained by introducing graded doses of norepinephrine. After a wash-out of 1 hr, the antagonist was introduced and the preparation left for 10 min before the second dose response curve to norepinephrine was recorded. It could be shown on a number of preparations that the curve obtained without antagonist during the second period was essentially the same as the first one in force development and in sensitivity to the agonist. When a pD_2 of 7·65 ± 0·11 was obtained for norepinephrine during the first period, a value of 7·59 ± 0·16 was recorded during the second one (n = 5, P > 0·5 by *t*-test).

(b) *Potassium-induced contractions (amiodarone only).* After a stabilization period of 90 min, depolarization was achieved by replacing the bathing fluid by high K^+ Krebs solution which was maintained until the contractile response was complete. The preparation was then returned to normal K^+ Krebs and amiodarone (40 μM) was added. After an incubation period of 10 min, the preparation was again depolarized by high K^+ Krebs.

Reagents

Modified Locke solution: 125 mM NaCl, 5·6 mM KCl, 2·16 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose.

Modified Krebs–bicarbonate solution (for norepinephrine test): 112 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 1·2 mM $MgSO_4$, 2·5 mM $CaCl_2$ and 11·5 mM glucose.

Modified Krebs–bicarbonate solution (for depolarization test): 112 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 1·2 mM $MgSO_4$, 1·26 mM $CaCl_2$ and 11·5 mM glucose.

High K^+ Krebs solution: 17 mM NaCl, 100 mM KCl, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 1·2 mM $MgSO_4$, 1·26 mM $CaCl_2$ and 11·5 mM glucose.

These solutions were prepared fresh daily with analytical grade reagents and distilled water. Drugs were obtained from commercial sources: isoproterenol hydrochloride (ICN), *l*-norepinephrine (Fluka), phentolamine (Ciba). *d,l*-Propranolol hydrochloride was prepared in our Chemical Department. Amiodarone hydrochloride (6·8 mg) was dissolved in 1 ml 50% ethanol (10 mM stock solution). 0·5 ml of this solution was added to 4·5 ml blood plasma (taken from the animal of which the organ was studied) and mixed slowly on a magnetic stirrer at room temperature. The final solution was obtained by adequate dilution with either Locke or Krebs. Actual concentration was measured by optical absorption at 242 nm against the appropriate blank.

Calculations

Means were compared statistically either by variance analysis (Snedecor's *F*-test) or by Student's *t*-test for paired values [6]. Regression lines were calculated by the least squares' method.

For the antagonists, competitive inhibition was characterized by the pA_2 value and non-competitive inhibition by the pD'_2 values, after Van Rossum [7].

RESULTS

Inhibition of the chronotropic effect of isoproterenol. The inhibition brought about by propranolol was clearly of the competitive type. A pA_2 value of 8·33 was obtained, in good agreement with the values obtained by others (8·35–8·80) [8].

The dose–response curves recorded in the presence of amiodarone are depicted in Fig. 1. The non-parallel shifting of the curves and the steady lowering of their respective maximum with increasing doses of the antagonist are characteristic of a non-competitive inhibition. Moreover, pD'_2 calculated for each concentration of amiodarone was essentially constant (Table 1) and the lowering of the maximum stimulation was dose-dependent, as shown on Fig. 2.

Roba [9] did not observe any significant effect on guinea-pig atria stimulated by norepinephrine, with 1 μg/ml (about 50 μM) amiodarone.

Table 1. Inhibition of the chronotropic effect of isoproterenol by amiodarone

Amiodarone concn (μM)	pD'_2 ± S.E.M. (n)	Snedecor's *F*
5	4·01 ± 0·11 (5)	
10	4·21 ± 0·09 (12)	1·13
20	4·23 ± 0·08 (6)	

P exceeds 0·05 for *F*-values below 3·49.

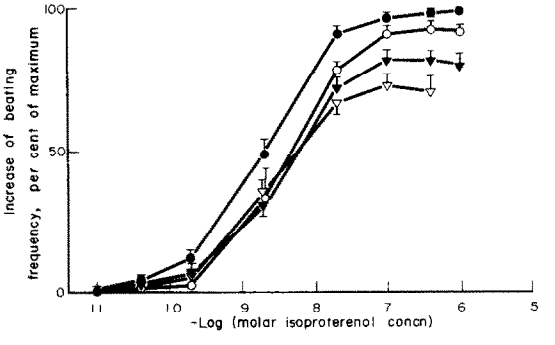

Fig. 1. Cumulative dose–response curves of the chronotropic effect of isoproterenol on spontaneously beating rabbit right atria in the presence of amiodarone. Amiodarone (molar) concentration: ● (no amiodarone), ○ (5×10^{-6}), ▼ (10^{-5}), ▽ (2×10^{-5}). Vertical bars represent standard error of the mean.

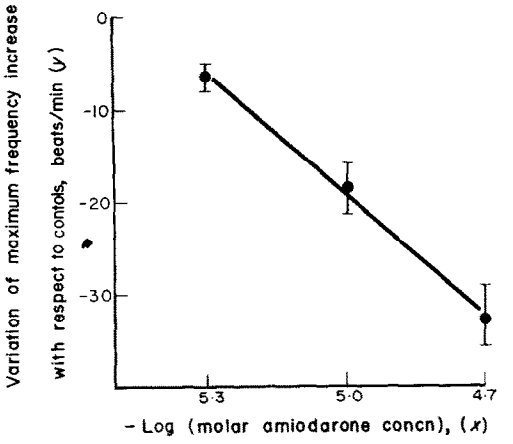

Fig. 2. Effect of amiodarone on the maximum frequency increase induced by isoproterenol in spontaneously beating rabbit right atria. Vertical bars represent S.E.M. Computed linear regression: $y = 44x - 239$.

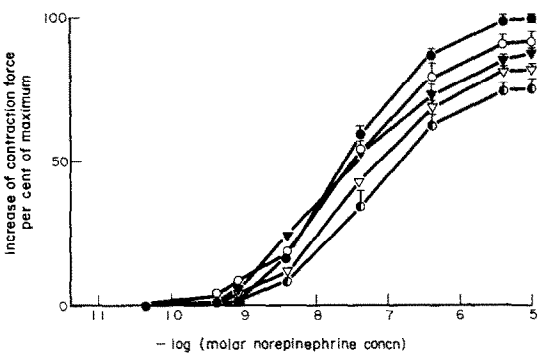

Fig. 3. Cumulative dose–response curves of norepinephrine-induced contractions of isolated rat aortic strips in the presence of amiodarone. Amiodarone (molar) concentration: ● (no amiodarone), ○ (5×10^{-6}), ▼ (10^{-5}), ▽ (2×10^{-5}), ◐ (4×10^{-5}). Vertical bars represent standard error of the mean.

Inhibition of norepinephrine-induced contractions of aortic strips. Phentolamine inhibited the norepinephrine-induced contractions in a typically competitive manner. A pA_2 value of 8·69 was computed, in good agreement with values obtained by others (7·94–8·69) [10].

Amiodarone decreased the slope and the maximum of the dose–response curves to norepinephrine in a dose-dependent way, as shown on Figs. 3 and 4. Hence, the antagonism was of the non-competitive type. The value of pD'_2 for each concentration of amiodarone was essentially constant (Table 2).

Roba [9] obtained essentially the same results for the inhibition by amiodarone of norepinephrine-induced contractions of *rabbit* aortic strips.

Effect of amiodarone on K^+ depolarization-induced contractions of aortic strips. Amiodarone 40 μM (i.e. at the highest concentration used in the previous section) did not significantly modify the contractile response to K^+ depolarization. The response recorded in the absence of amiodarone reached 98·7 ± 1·8 per cent of the initial maximum contraction (mean ± S.D., n = 3). In the presence of amiodarone, contractions reached a mean of 94·7 ± 2·0 (n = 4). With a Student's *t*-value of 1·41, this slight difference was not significant.

DISCUSSION

The adrenergic antagonism displayed by amiodarone could not be ascribed to an interaction with calcium transport across the plasmic membrane, at least in the aortic preparation. It was however of the non-competitive type in the experiments reported above. This result implies that amiodarone does not compete with the agonists at their respective recognition sites. Such a conclusion was not unexpected in view of the fact that amiodarone antagonizes different types of agonists: β_1-adrenergic agonists like isoproterenol, α-adrenergic agonists like norepinephrine, and also glucagon [2]. Competition with such widely different agents would have been highly improbable.

It can be assumed, then, that amiodarone acts beyond the step of hormone binding to receptor site, somewhere on the chain of events leading to the observed pharmacological activation.

It may be speculated that the point of action should be on a pathway common to α-adrenergic activation in vascular smooth muscle, β_1-adrenergic stimulation on the heart pacemaker cells, and glucagon chronotropic activation of the heart. All these stimulations are in fact cAMP-dependent [11]. It has been shown on plasmic membranes from rat myocardium that amiodarone antagonizes hormone-dependent adenylate cyclase stimulation, but that the drug does not inhibit the basal adenylate cyclase activity nor its acti-

Table 2. Inhibition of the norepinephrine-induced contractions of aortic strips

Amiodarone concn (μM)	pD'_2 ± S.E.M.	(n)	Snedecor's *F*
5	4·01 ± 0·16	(4)	1·25
10	4·17 ± 0·05	(6)	
20	4·03 ± 0·08	(5)	
40	3·91 ± 0·06	(4)	

P exceeds 0·05 for *F* values under 3·29.

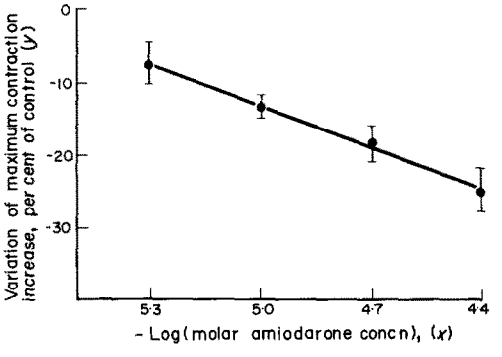

Fig. 4. Effect of amiodarone on the maximum contraction increase induced by norepinephrine in isolated rat aortic strips. Vertical bars represent S.E.M. Computed linear regression: $y = 19x - 108$.

vation by fluoride.* The available evidence implies that amiodarone would act between the hormone binding step and the subsequent activation of membrane-bound adenylate cyclase, i.e. within the membrane itself. To our knowledge, no other drug antagonizes hormone action at such an early stage without interfering with the receptor site itself.

In this respect, it may be important to recall that amiodarone displays tissular specificity. Adrenergic (or glucagon) antagonism has been shown only on the heart and the arteries [1, 2]. The drug has no action on epinephrine-induced lipolysis of the epididymal fat pad [12], on catecholamine- or glucagon-induced activation of rat liver adenylate cyclase *in tro*,* on the *in vivo* epinephrine-induced serum lactic acid and free fatty acid increase in the rat [12], nor on the bronchodilatation triggered by isoproterenol in guinea-pigs.† It seems therefore that amiodarone interacts only with the membranes of some tissues.

* J. Broekhuysen and M. Ghislain, unpublished results.
† R. Charlier, unpublished results.

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