

Short Communication

High Affinity of Carazolol for β -Adrenoceptors Coupled to the Adenylyl Cyclase in Ventricular Myocardium of Kitten and *Xenopus laevis*

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Summary. Catecholamine-induced stimulation of adenylyl cyclase in ventricular membranes of kitten and *Xenopus laevis* was antagonized competitively by carazolol. Apparent equilibrium constants ranging between 100–170 pM were estimated for the β -adrenoceptor-carazolol complex.

Key words: High affinity carazolol- β -adrenoceptor – Complex heart.

Introduction

Carazolol exhibits very high and similar affinity for β -adrenoceptors mediating positive chronotropic and inotropic effects of (–)-isoprenaline in isolated heart preparations of kitten, guinea pig and rat. The apparent equilibrium dissociation constant K_B of the β -adrenoceptor-carazolol complex in these systems was found to range from 100–200 pM (Lemoine and Kaumann, 1978). We report here the nature of the antagonism by carazolol of catecholamine-induced stimulation of heart adenylyl cyclase.

Some differences of heart β -adrenoceptors of rat and *Xenopus laevis* were detected by comparing the affinities of certain ligands in both species (Kaumann, 1977). It was found that practolol exhibited significantly lower affinity for *Xenopus* β -adrenoceptors than for rat adrenoceptors; the converse was observed with the selective ligand H35/25 (Carlsson et al., 1972). We have seen that kitten and *Xenopus* heart β -adrenoceptors also differ in their affinity characteristics for H35/25 and practolol (unpublished experiments). To inquire whether or not carazolol is another ligand that preferentially binds more to the β -adrenoceptors of

one species than to those of another, its affinity for cyclase-coupled β -adrenoceptors was compared in ventricular membranes of kitten and *Xenopus*.

Methods

To avoid β -adrenoceptor-occupancy with endogenous catecholamines (Kaumann and Birnbaumer, 1974), kittens (weighing 0.8–1.5 kg) and toads (weighing 90–150 g) were pretreated with 5 mg/kg s.c. and 50 mg/kg i.m. reserpine, respectively, 24 h before sacrificing. These doses of reserpine cause nearly complete depletion of noradrenaline in cat heart (Lee and Shideman, 1959) and of adrenaline in *Xenopus* heart (Strobach, personal communication). Membrane particles of kitten ventricle were prepared as before (Kaumann and Birnbaumer, 1974). Ventricular membrane particles of *Xenopus* were prepared as for kitten ventricle, except that the ventricle was dissected in solution containing (mM):

NaCl 102.5, KCl 2.7, NaHCO₃ 1.2, and CaCl₂ 1.3 in deionized, redistilled water. Experiments were carried out on membranes which had been stored at –70°C.

In order to obtain the highest possible stimulation of the adenylyl cyclase activity by catecholamines, various incubation conditions were tried. Maximum increases of adenylyl cyclase activity over basal activity with catecholamines were observed with GTP in kitten membranes and 5'-guanylylimidodiphosphate in *Xenopus* membranes. For other incubation conditions see legend to Figure 1. In order to measure [³²P]cAMP membranes were incubated with [α -³²P]ATP; [³H]cAMP was added as a recovery marker. The [³²P]cAMP formed and the [³H]cAMP were isolated by the method of Salomon et al. (1974) and determined by liquid scintillation counting. Protein was determined with the method of Lowry et al. (1951). A salt of (±)-carazolol (Boehringer, Mannheim) was prepared with tartaric acid.

Results and Discussion

Carazolol (0.4–60 nM) did not significantly change the basal activity of adenylyl cyclase in kitten and *Xenopus* membranes (left abscissa of Fig. 1A and B). However, 0.4–60 nM carazolol caused nearly parallel and nearly surmountable shifts of concentration-effect curves for (–)-isoprenaline in both species, suggesting competitive inhibition. Analysis for competitive be-

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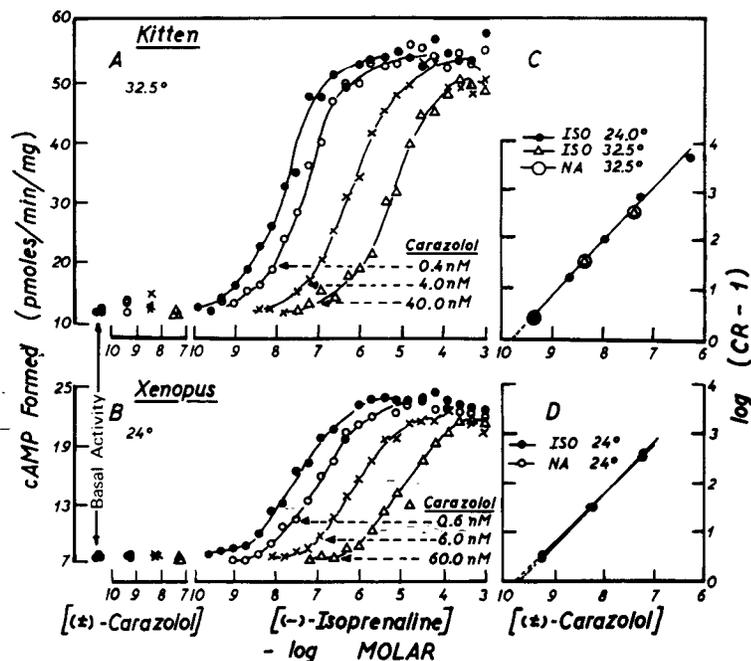


Fig. 1 A and B. Surmountable antagonism by carazolol of the (-)-isoprenaline-induced stimulation of adenylyl cyclase in ventricular membranes of kitten at 32.5° C (A) and toad at 24° C (B). Each symbol represents the result from a single determination. Membrane particles were incubated for 10 min in 50 μ l of medium containing 0.1 mM [α - 32 P]ATP (specific activities 125 cpm/pmole and 194 cpm/pmole in A and B, respectively), 2.0 mM MgCl₂, 1.0 mM EGTA, 0.1 mM ascorbic acid, 1 mM MgCl₂, 25 mM Tris-HCl, pH 7.5, indicated concentrations of (-)-isoprenaline and (±)-carazolol and either 10 μ l GTP (panel A) or 10 μ M 5'-guanylimidodiphosphate (panel B). The reactions were stopped by addition of 100 μ l of a solution containing 10 mM cAMP, 40 mM ATP and 1% sodium dodecyl sulphate, followed by immediate boiling for 3.5 min. Protein was 55.5 μ g and 26.5 μ g per assay for A and B, respectively. C and D. Simple competitive antagonism of (±)-carazolol against the effects of catecholamines. Equieffective concentration ratios (CR) of (-)-isoprenaline (closed circles) were taken from A and B and represented double-logarithmically as a function of (±)-carazolol concentration in C and D, respectively. Other symbols were from additional experiments with (-)-noradrenaline carried out at the temperature indicated on the figure

Table 1. Apparent equilibrium dissociation constants K_B^a for carazolol- β -adrenoceptor complexes in ventricular membranes of kitten and *Xenopus*

Species	Agonist	Temperature °C	N ^b	Slope of Schild plot \pm S.D.	$-\log M K_B \pm 2$ S.D.
Kitten	(-)-isoprenaline	32.5	3	1.08 \pm 0.07	9.87 \pm 0.22
Kitten	(-)-noradrenaline	32.5	3	1.06 \pm 0.06	9.89 \pm 0.18
Kitten	(-)-isoprenaline	24	5	1.08 \pm 0.06	9.98 \pm 0.30
<i>Xenopus</i>	(-)-isoprenaline	24	3	1.05 \pm 0.03	9.78 \pm 0.10
<i>Xenopus</i>	(-)-noradrenaline	24	3	1.00 \pm 0.03	9.79 \pm 0.06

^a $K_B = [\text{Carazolol}] (\text{CR}-1)$; CR is EC₅₀ ratio of catecholamine in the presence and absence of carazolol

^b N = Number of concentration ratios of catecholamine

behaviour was made with Schild plots as shown in Figure 1C and D (Arunlakshana and Schild, 1959). Slopes of regressions of log (EC₅₀ concentration-ratio of (-)-isoprenaline-1) against log [(±)-carazolol] were close to 1.0 in both species (Table 1) as expected from simple competitive antagonism. Concentration-effect curves for (-)-noradrenaline were also antagonized competitively by carazolol in both species (experiments not shown) yielding Schild plots with slopes that were also not significantly different from 1.0 (Fig. 1C and D, Table 1). Carazolol antagonized competitively the effect of (-)-isoprenaline both at 24° C and at 32.5° C (Fig. 1A and C and Table 1).

Because of the simple competitive nature of the antagonism of the effects of catecholamines by car-

azolol, equilibrium constants K_B were calculated (Table 1). The K_B of the carazolol- β -adrenoceptor complex was 100–170 pM in both kitten and *Xenopus* membranes. The affinity of carazolol for myocardial β -adrenoceptors was the same, regardless of species (kitten vs *xenopus*), temperature (24 vs. 32.5° C, kitten only) or agonist [(-)-isoprenaline vs. (-)-noradrenaline]. The K_B of carazolol for adenylyl-cyclase-coupled β -adrenoceptors agrees with its K_B of 100–200 pM estimated for β -adrenoceptors mediating positive chronotropic and inotropic effects of catecholamines in mammalian heart (Lemoine and Kaumann, 1978).

Unlike the selective ligands H35/25 (Carlsson et al., 1972) and practolol which exhibit preferential affinities

for *xenopus*- and mammalian- β -adrenoceptors, respectively (Kaumann, 1977), carazolol was non-selective in this respect.

Comparison of the K_B of carazolol with K_B 's reported for other high affinity ligands reveals that the affinity of carazolol for adenylyl cyclase-coupled β -adrenoceptors is about 5 and 20 times higher than that of bupranolol and propranolol, respectively (Kaumann and Birnbaumer, 1974). In our experience carazolol is so far the ligand with the highest affinity for cardiac β -adrenoceptors. Radioactive and fluorescent carazolol analogues may serve as promising ligands for the elucidation of properties of myocardial and perhaps other β -adrenoceptors.

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