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A Comparison of the Influence of N-Isopropyl and N-Tert Butyl Substituents on the Affinity of Ligands for Sinoatrial β-Adrenoceptors in Rat Atria and β-Adrenoceptors Coupled to the Adenylyl Cyclase in Kitten Ventricle

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Summary. The potencies as β-adrenoceptor blockers of KL 1006 (the N-isopropyl analogue of bupranolol), pindolol, propranolol and carazolol were compared with potencies of their corresponding *tert.* butyl analogues bupranolol, tert. butylpindolol, tert. butylpropranol and ter. butylcarazolol.

Experiments were carried out at 32.5°C in rat right atria – assessing β-adrenoceptor dependent chronotropic effects – and kitten ventricular membranes – assessing β-adrenoceptor dependent stimulation of adenylyl cyclase – from reserpine pretreated animals.

1. The effects of (–)-isoprenaline on these two systems were antagonized competitively by each of the 8 ligands.

2. None of the 8 ligands caused stimulation or depression of basal adenylyl cyclase activity at concentrations causing antagonism of the effects of (–)-isoprenaline.

3. Pindolol, tert. butylpindolol, carazolol and tert. butylcarazolol were weak partial agonists in rat atria. concentrations of these ligands for producing significant stimulation were higher than those causing significant antagonism of the chronotropic effects of (–)-isoprenaline.

4. Bupranolol, tert. butylpindolol, tert. butylpropranolol and tert. butylcarazolol were 6.6, 3.0, 3.2 and 1.1 times more potent as β-adrenoceptor antagonists in rat atria than their corresponding N-isopropyl analogues, respectively.

5. Bupranolol, tert. butylpindolol, tert. butylpropranolol and tert. butylcarazolol were 4.8, 3.0, 2.7 and 0.6 times more potent as β-adrenoceptor antagonists in kitten membranes than their corresponding N-isopropyl analogues, respectively.

6. The enhanced affinity for myocardial β-adrenoceptors of N-tert. butyl ligands (with respect to the affinity of the corresponding N-isopropyl ligands) appears to be inversely correlated with the size of the ring of the ligands.

Key words: N-isopropyl and N-tert. butyl ligands – β-Adrenoceptor-affinity – Heart ventricular adenylyl cyclase – Rat sinoatrial node.

Introduction

Since it was established that isoprenaline is more potent than either adrenaline or noradrenaline as agonists for myocardial β-adrenoceptors (Ahlquist, 1948), N-isopropyl derivatives were preferentially developed as β-adrenoceptor antagonists (Powell and Slater, 1958; Moran and Perkins, 1958; Black and Stephenson, 1962; Black et al., 1964 and 1965; Åblad et al., 1967; Brunner et al., 1968; Giudicelli et al., 1969). With the aim of obtaining tissue-selective β-adrenoceptor antagonists with high affinity, several N-butyl (N-t-butyl) derivatives were also introduced more recently (Kaumann et al., 1970; Kaplan et al., 1971; Nakanishi et al., 1972; Schweiger et al., 1972; Crowther et al., 1972; Scriabine et al., 1973; Meyer et al., 1973; Fothergill et al., 1975; Weinstock et al., 1976). However, it is not clear from these studies whether the N-t-butyl of these compounds actually does enhance affinity for the β-adrenoceptors because comparisons were usually not made with the corresponding N-isopropyl ligands. Thus, comparisons of affinities were made for several compounds either under in vivo conditions (Cheymol et al., 1972; Levy, 1973; Femmer et al., 1975) or for a single compound in only one in vitro system (Mylecharane and Raper, 1971).

Early studies by Marsh et al. (1948) indicated that the agonist N-t-butylnoradrenaline was more potent than isoprenaline in several β-adrenoceptor responsive

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systems. Unfortunately, potencies of agonists modifying a function of a tissue do not necessarily relate to the affinity of the agonists for the receptors of that tissue. The present study was undertaken to examine systematically the influence of amino substituents on the affinity of ligands for heart β-adrenoceptors. To this end, we studied in two in vitro systems the blocking ability of four high affinity β-antagonists by comparing the N-isopropyl derivative of each with its corresponding N-t-butyl analogue.

High affinity ligands were chosen to avoid interference of toxic actions with affinity estimations. Affinity of each of the selected ligands was determined for sinoatrial β-adrenoceptors in rat atria (because this system seems to have a homogeneous β-adrenoceptor population; Kaumann, 1977a) and for β-adrenoceptors coupled to the adenylyl cyclase system of kitten ventricular membranes.

Methods

Isolated Atria. Experiments were carried out at 32.5°C on spontaneously beating right atria of Wistar II rats (either sex, 180–320 g) pretreated with 5 mg/kg reserpine (18–24 h). The rats were sacrificed by a blow on the head and the hearts rapidly removed and dissected at room temperature in oxygenated salt solution. The solution contained (mM): Na^+ 140; K^+ 5; Ca^{2+} 2.25; Mg^{2+} 1; Cl^- 98.5; SO_4^{2-} 1; HCO_3^- 29; HPO_4^{2-} 1; fumarate 10; pyruvate 5; L-glutamate 5; glucose 10; ethylenediamine tetraacetic acid disodium salt (EDTA) 0.04. The solution was prepared with deionized, glass-redistilled water and equilibrated with 95% O_2 and 5% CO_2. Atria were set up in pairs in an isolated organ bath (50 ml) and suspended at enough resting tension for measurable development of tension. Equilibrium chronotropic effects of drugs were expressed as mean ± SEM beats/min. Cumulative concentration-effect curves for β-adrenoceptor ligands were determined by the sequential addition of drugs to the isolated organ bath in amounts that increased the total concentration in steps of about 1/2 log unit.

Membrane Particles. Membranes were prepared from hearts of reserpine-pretreated (5 mg/kg, 18–24 h) kittens (either sex, 0.6–1.4 kg) as described by Kaumann and Birnbaumer (1974). Membrane particles were stored at −70°C until used.

Adenylyl Cyclase Assay. Membrane particles (50–75 µg protein) were incubated for 10 min at 32.5°C in 50 µl of incubation medium containing: 0.1 mM [α-^{32}P]ATP (specific activities between 145–1469 cpm/pmole), 2.0 mM $MgCl_2$, 10 µM GTP, 1.0 mM EGTA, 0.1 mM ascorbic acid, 1 mM [^{3}H]cyclic AMP (6,000–12,000 cpm; added both to monitor recovery and to competitively inhibit endogenous phosphodiesterase activity), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase (Calbiochem), 0.1 mg/ml myokinase (Sigma), 25 mM Tris-HCl, pH 7.5 and varying concentrations of (−)-isoprenaline (see Fig. 6) with and without β-adrenoceptor blocking ligand as indicated. The cyclic AMP formed was separated by the method of Salomon et al. (1974) and quantitated by liquid scintillation counting. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Drugs. For comparison of N-isopropyl ligands with N-t-butyl ligands, the following racemates were used (formulae are depicted in Fig. 7):

Pindolol, 1-(indol-4-yloxy)-3-isopropylamino propan-2-ol tartrate (Sandoz); tert. butylpindolol, 1-(indol-4-yloxy)-3-tert. butylamino propan-2-ol tartrate (Sandoz); bupranolol (KL 255), 1-(2-chloro-5-methylphenoxy)-3-tert. butylamino propan-2-ol · HCl (Sanol, Monheim); KL 1006, 1-(2-chloro-5-methylphenoxy)-3-isopropylamino propan-2-ol · HCl (Sanol, Monheim); propranolol, 1-(naphth-1-yloxy)-3-isopropylamino propan-2-ol · HCl (ICI); tert. butylpropranolol, 1-(naphth-1-yloxy)-3-tert. butylamino propan-2-ol · HCl (ICI); carazolol (BM 51052), 1-(carbazol-4-yloxy)-3-isopropylamino propan-2-ol · HCl (Boehringer, Mannheim); tert. butyl carazolol, 1-(carbazol-4-yloxy)-3-tert. butylamino propan-2-ol · HCl (Boehringer, Mannheim). Other drugs used were (−)-isoprenaline bitartrate (Sterling Winthrop) and reserpine phosphate (Serpasil, Ciba-Geigy).

Statistics and Evaluation of Drug-Receptor Constants. When ligands were pure antagonists, apparent equilibrium dissociation constants (K_B) for antagonist-receptor complexes were calculated under equilibrium conditions from the relation

$$K_B = \frac{[\text{antagonist}]}{\text{concentration ratio} - 1}. \quad (1)$$

Concentration ratios were measured from normalized concentration-effect curves for (−)-isoprenaline at the EC_{50} level. The estimation of K_B values from concentration ratios depends on the assumption that the antagonism is a simple competitive one. As a check of that assumption we constructed a least square plot of log (concentration ratio of (−)-isoprenaline −1) as a function of log antagonist concentration (Arunlakshana and Schild, 1959; AS plot). A slope of one of AS plots is expected from simple competition.

When ligands were partial agonists (P), apparent equilibrium dissociation constants K_P for the partial agonist-receptor complexes were calculated under equilibrium conditions from the relation

$$K_P = \frac{[P]}{y_P} - [P] \quad (2)$$

where [P] is the concentration of P used and y_P is the fractional receptor occupancy at that [P]. y_P was estimated from a plot of equieffective concentrations of (−)-isoprenaline in the absence (A_2) and presence (A_3) of [P]. A weighted linear regression was calculated according to the relation

$$A_2 = \text{intercept} + \text{slope} \cdot A_3 \quad (3)$$

fitted with weights $([A_3] + [EC_{50} \text{ of } A_3])^{-2}$ and y_P calculated from 1 − slope (Marano and Kaumann, 1976). This method for calculating K_P is based on the assumption that spare receptors (Stephenson, 1956) exist for the full agonist, but not for the partial agonist, i.e. that full agonists require occupancy of only a small fraction of adrenoceptors to elicit a maximal response and that maximal effects of partial agonists can only be elicited upon saturation of receptors. Recent evidence in heart muscle (Kaumann, 1978) and cardiocytes (Kaumann and Bojar, 1978) supports this assumption.

Significance of the difference between 2 mean log K_B's (or K_P's) was determined using Student's t-test. The difference was considered significant at $P \leq 0.01$.

Results

The Effects of N-Isopropyl and N-t-butyl Ligands on Rat Atria

KL 1006 and propranolol and their corresponding N-t-butyl analogs, bupranolol and tert. butylpropranolol,

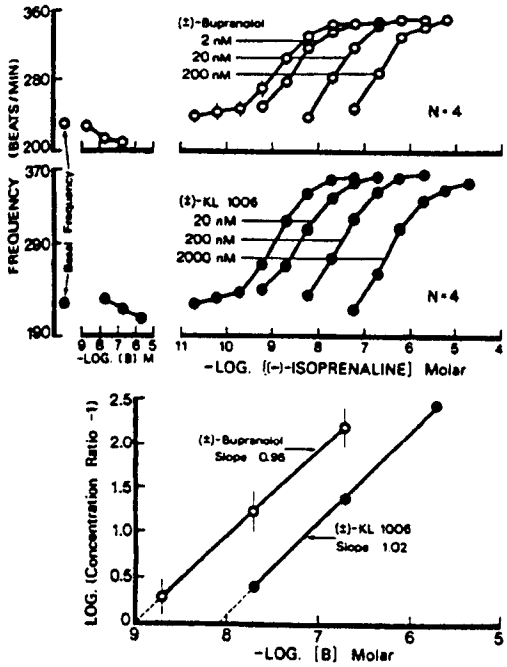

Fig. 1. Comparison of the antagonism by bupranolol and KL 1006 of the positive chronotropic effects of (–)-isoprenaline in rat atria at 32.5° C. A control concentration-effect curve for (–)-isoprenaline was followed by successive curves determined in the presence of antagonist. At the end of each concentration-effect curve, the atria were washed repeatedly and the antagonist for the next curve was added immediately. Any given concentration of antagonist was left in contact with the tissue for 1 h before the corresponding concentration-effect curve was begun. Frequencies with each antagonist concentration immediately before commencing a curve for (–)-isoprenaline are shown on the left abscissa of the upper and middle panel. Concentration ratios measured at the EC_{50} level from the curves in the upper and middle panel were used to construct the AS plots of the lower panel. Vertical lines through symbols are SEM; symbols without lines have smaller SEM than size of symbol. *N*, number of tissues

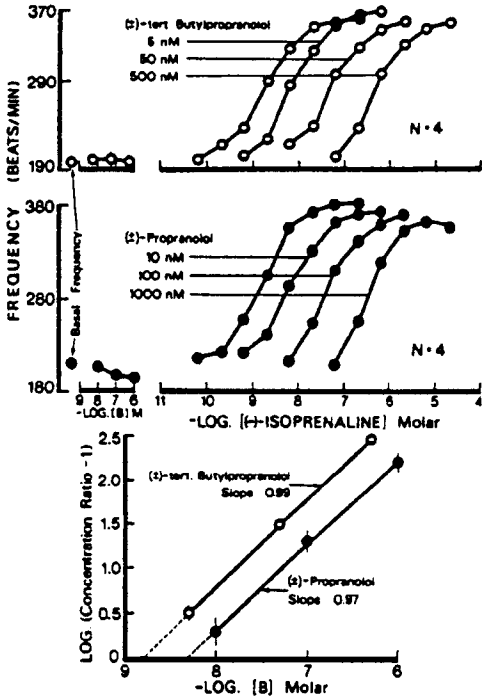

Fig. 2. Comparison of the antagonism by tert. butylpropranolol and propranolol of the positive chronotropic effects of (–)-isoprenaline in rat atria. Experimental conditions and results are plotted as in Fig. 1

antagonized surmountably the positive chronotropic effects of (–)-isoprenaline. Concentration-effect curves for (–)-isoprenaline were shifted in nearly parallel fashion to the right. The AS plots constructed with EC_{50} ratios for (–)-isoprenaline in the presence and absence of the antagonists yielded slopes that did not differ significantly from unity (Figs. 1 and 2), suggesting simple competitive antagonism. An estimate of the equilibrium dissociation constants (K_B) was derived for each compound (see Table 1). Bupranolol and tert. butylpropranolol have affinities 6.6- and 3.2-fold higher than their respective N-isopropyl anlogs, KL 1006 and propranolol.

Both pindolol and tert. butylpindolol elicited concentration-dependent increases in beating rate (Fig. 3). Both ligands, at 0.6 μM, caused nearly maximal increases in beating rate with approximate intrinsic activities [with respect to the maximum effect of (–)-isoprenaline] of 0.26 and 0.25 for pindolol and tert. butylpindolol, respectively. Both ligands also induced pronounced and completely surmountable rightward shifts of the concentration-effect curves for (–)-isoprenaline. It was assumed that pindolol and tert. butylpindolol are partial agonists and their apparent equilibrium constants were calculated as by Marano and Kaumann (1976). The affinity for sinoatrial β-adrenoceptors of tert. butylpindolol was determined to be 3.0 times higher than the affinity of pindolol (Table 1). Both pindolol and tert. butylpindolol caused positive chronotropic effects only at receptor occupancies greater than 50% (Fig. 3) under the present conditions.

Both carazolol and tert. butylcarazolol caused surmountable and nearly parallel shifts in the concentration-effect curves of (–)-isoprenaline (Fig. 4). The AS plots derived for carazolol and tert. butylcarazolol exhibited slopes of 0.90 and 1.03, respectively. The reason(s) for the slight departure from a unit slope with carazolol are not understood. Because the departure is small and since in our previous work with this compound (Morris et al., 1978) an AS plot of unit slope was obtained, a simple, competitive antagonism was assumed for carazolol as well. The estimates of the blocking constants for carazolol and tert. butylcara-

Table 1. Apparent equilibrium dissociation constants (−log M, mean ± SEM) for β-adrenoceptor-drug complexes of N-t-butyl and N-isopropyl ligands

	Rat atria Sinoatrial node	P	Kitten ventricle Adenylyl cyclase	P
Bupranolol	8.94 ± 0.08 (4)[a]	<0.001	9.07 ± 0.03 (4)[b]	<0.001
KL 1006	8.12 ± 0.05 (4)		8.39 ± 0.02 (4)	
Tert. butylpindolol	9.26 ± 0.04 (4)	<0.001	9.52 ± 0.02 (3)	<0.01
Pindolol	8.79 ± 0.07 (4)		9.04 ± 0.07 (4)	
Tert. butylpropranolol	8.79 ± 0.06 (4)	<0.001	9.07 ± 0.05 (3)	<0.01
Propranolol	8.28 ± 0.07 (4)		8.63 ± 0.08	
Tert. butylcarazolol	9.82 ± 0.10 (4)	>0.05	9.65 ± 0.04 (3)	>0.02
Carazolol	9.91 ± 0.07 (12)		9.87 ± 0.07 (3)	

[a] No. of tissues between parentheses
[b] No. of concentration ratios between parentheses

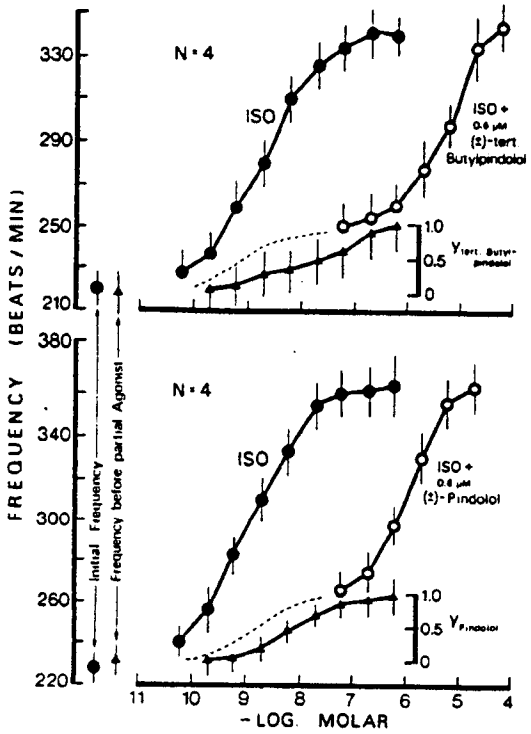

Fig. 3. Comparison of tert. butylpindolol and pindolol as stimulants and as antagonists against the effects of (−)-isoprenaline in rat atria. Three cumulative concentration-effect curves were determined. After the first curve [for (−)-isoprenaline] the agonist was washed out. At the end of the second curve (upper panel for tert. butylpindolol and lower panel for pindolol) the highest concentration was left in the bath so that the third curve [for (−)-isoprenaline] was determined in the presence of 0.6 μM of either tert. butylpindolol or pindolol. Fractional receptor occupancies (y) (broken lines) were estimated as described under Methods. Each concentration of the partial agonists was 30 min in contact with the atria, except 0.6 μM which was left for 60 min before starting the curve for isoprenaline. For other details see Fig. 1

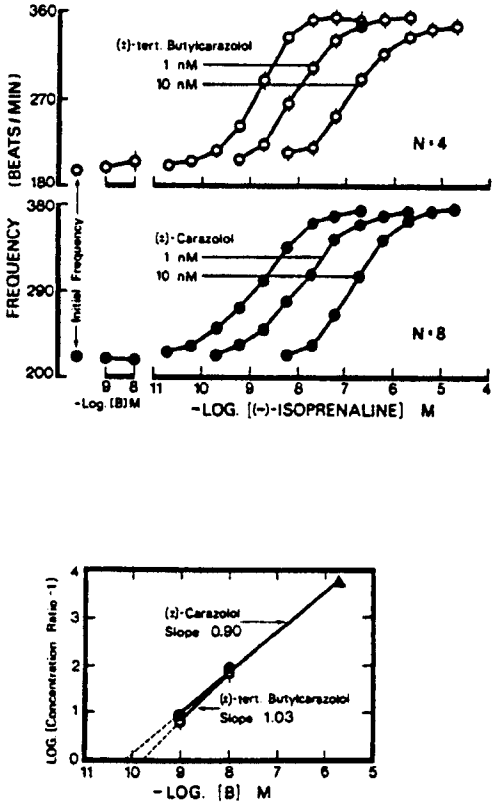

Fig. 4. Comparison of the antagonism by tert. butylcarazolol and carazolol of the positive chronotropic effects of (−)-isoprenaline in rat atria. A control concentration-effect curve for (−)-isoprenaline was followed by 2 successive curves determined in the presence of antagonist. The 2 concentrations of antagonist were 150 min in contact with the tissue before the next curve for (−)-isoprenaline was begun. Lower panel shows AS plots (circles) from the experiments of the upper two panels. The AS plot for carazolol includes a point (triangle) from a separate experiment ($N = 4$) with 1 μM carazolol (not shown). For other details see legend to Fig. 1

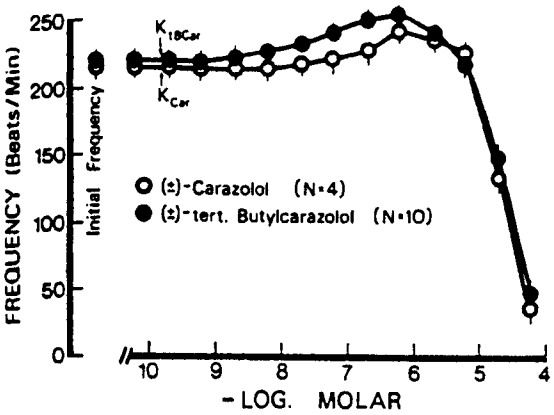

Fig.5. Stimulant and depressant effects of carazolol and tert. butylcarazolol on rat atria. Cumulative concentration-effect curves determined at 32.5°C with intervals between additions as follows: if no effect, 15 min; if drug had a detectable effect within 15 min, time was allowed for full development of the effect except that the time was arbitrarily limited to 30 min during the phase of depression. K's indicate mean K_B's estimated from the experiments of Fig.4

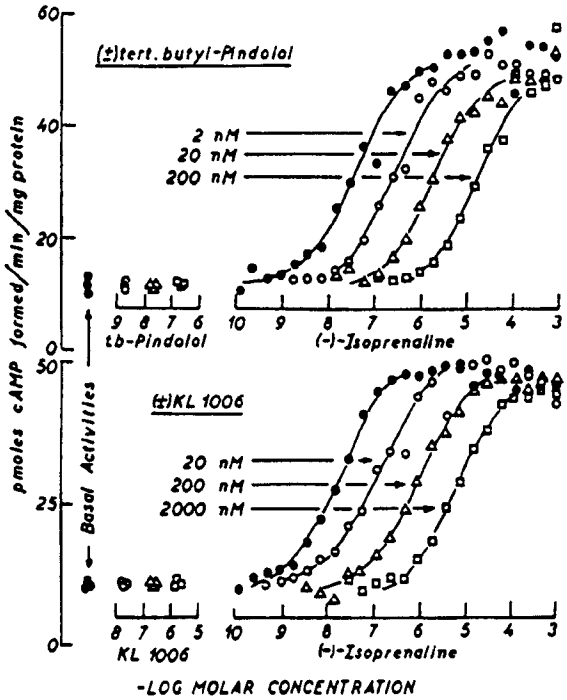

Fig.6. Surmountable antagonism by tert. butylpindolol (tb-pindolol) and KL 1006 (the N-isopropyl analogue of bupranolol) of the (−)-isoprenaline-induced stimulation of adenylyl cyclase in kitten ventricular membranes at 32.5°C. Each symbol is from a single determination. For incubation conditions see Methods. Specific activity of [α-^{32}P]ATP was 145 cpm/pmole and 1469 cpm pmole for the experiments with tert. butylpindolol and KL 1006, respectively. AS plots with data from these and other experiments are shown in Fig.7

zolol are not significantly different (Table 1). The highest concentration of tert. butylcarazolol used for blockade (10 nM) appeared to cause a marginal increase in the beating rate (Fig. 4). Therefore, complete concentration-effect curves for tert. butylcarazolol and of carazolol (for comparison) were determined. Both ligands caused positive chronotropic effects at relatively high concentrations. The EC_{50}'s for carazolol and tert. butylcarazolol for enhancement of beating rate were found to be 2.9 and 2.4 log units higher than their respective K_B's (Fig. 5).

Apparent Affinities of N-Isopropyl and N-t-butyl Ligands for β-Adrenoceptors Coupled to the Adenylyl Cyclase of Kitten Ventricular Membranes

Figure 6 shows that tert. butylpindolol and KL 1006 did not modify basal adenylyl cyclase activity at concentrations causing parallel and surmountable shifts of concentration-effect curves for (−)-isoprenaline. Similarly, propranolol, tert. butylpropranolol, carazolol, tert. butylcarazolol, bupranolol and pindolol did not change basal adenylyl cyclase activity at blocking concentrations (data not shown). Slopes of AS plots derived for all eight ligands were not significantly different from unity. Experiments with some of the ligands used in the present study had already been examined under adenylyl cyclase assay conditions of 2 mM ATP without GTP addition (Kaumann and Birnbaumer, 1974). Our present incubation condition with 0.1 mM ATP and 10 μM GTP did not change the degree of antagonism produced by pindolol, propranolol or bupranolol against (−)-isoprenaline (Fig. 7). K_B's were estimated for all eight ligands (Table 1). It is apparent that bupranolol, tert. butylpropranolol and tert. butylpindolol display a higher affinity for the β-adrenoceptors coupled to the adenylyl cyclase than their corresponding N-isopropyl analogs. On the other hand, the affinity of tert. butylcarazolol is not significantly different from the affinity of carazolol.

Discussion

Our estimates of K_P for pindolol and tert. butylpindolol were calculated with the method of Marano and Kaumann (1976) under the spare receptor (Stephenson, 1956) assumption: All used concentrations of (−)-isoprenaline (in the absence and presence of either partial agonist) should be smaller than the equilibrium dissociation constant K_{ISO} for the (−)-isoprenaline-β-adrenoceptor complex. While this assumption may well be true for all used concentrations of (−)-isoprenaline in the absence of partial agonist, it is less realistic for the high concentrations of (−)-isoprenaline ($> 10^{-6}$ M)

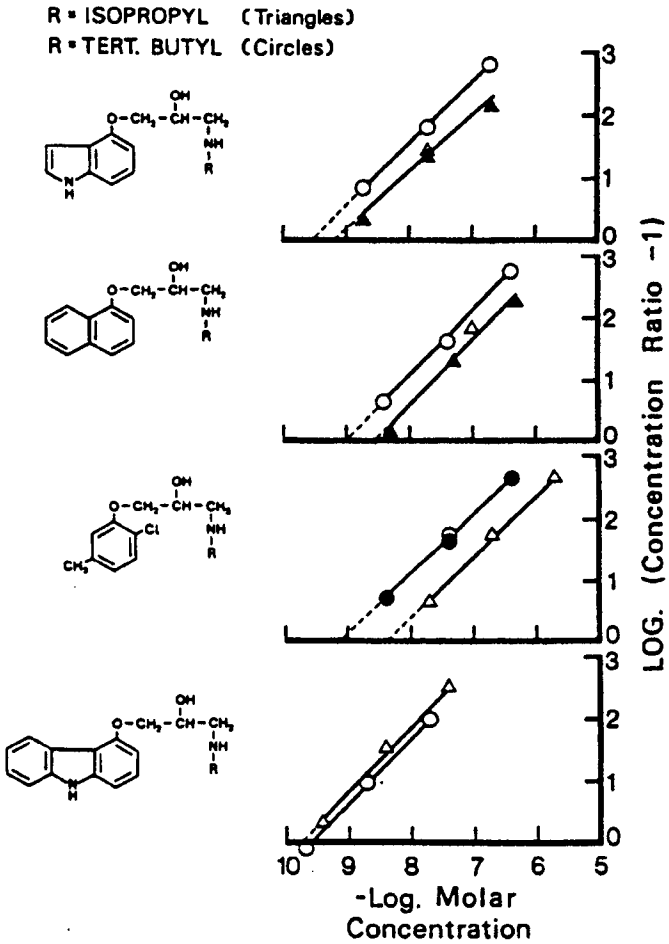

Fig. 7. Simple competitive antagonism by N-isopropyl and tert. butyl ligands against the adenylyl cyclase stimulating effects of (–)-isoprenaline in kitten ventricular membranes at 32.5°C. These AS plots were derived from the experiments in Fig. 6 and from additional experiments (not shown). The formulae (with the N-isopropyl substituent) represent from top to bottom: pindolol, propranolol, KL 1006 and carazolol. Open symbols are from experiments carried out with 0.1 mM ATP and 10 μM GTP (see Methods); closed symbols from experiments with 2 mM ATP (no GTP added). Experiments with pindolol and propranolol with 2 mM ATP were those of Kaumann and Birnbaumer (1974). Slopes of AS plots were (mean ± S.D.): 0.90 ± 0.10 for pindolol, 0.99 ± 0.02 for tert. butylpindolol, 1.07 ± 0.13 for propranolol, 1.06 ± 0.05 for tert. butylpropranolol, 1.00 ± 0.04 for KL 1006, 0.97 ± 0.05 for bupranolol, 1.08 ± 0.07 for carazolol and 1.06 ± 0.03 for tert. butylcarazolol

used in the presence of either partial agonist (Fig. 3). This would suggest that the method of Marano and Kaumann does not seem suitable for the estimation of K_P from the experiments of Fig. 3.

The estimates of K_P with that method are, however, reliable approximations to the actual K_P for the following reasons: The affinity of (–)-isoprenaline, as measured from direct binding on heart β-adrenoceptors (Kaumann, 1978), is at least 100 times lower than the affinities of pindolol and tert. butyl pindolol for β-adrenoceptors coupled to the adenylyl cyclase (Table 1, this report). In contrast, as seen in rat atria, (–)-isoprenaline is not only one order of magnitude more potent than either partial agonist, but causes also a 4 times greater increase in beating rate. Therefore, the efficacy of (–)-isoprenaline must be considerably greater than the efficacy value of either of the partial agonists. If the efficacy of full agonist greatly exceeds the efficacy of partial agonist, an estimate of K_P is reliable (Kenakin and Black, 1978) from the regression of equieffective concentrations of A_2 vs. A_3 (equation 3 of Methods, present report).

Our estimates of affinities for each of the tested N-isopropyl ligands and N-t-butyl ligands are similar (within 0.35 log units or less) for β-adrenoceptors of rat sinoatrial node and for kitten ventricular β-adrenoceptors coupled to the adenylyl cyclase. Furthermore, the K_B's (or K_P's) of this report do not differ significantly from our previous estimates in various heart systems [for bupranolol, Kaumann (1972, 1977b), Kaumann and Birnbaumer (1974), Kaumann et al. (1977); for propranolol, Kaumann and Blinks (1967), Kaumann and Birnbaumer (1974); for pindolol, Kaumann (1973), Kaumann and Birnbaumer (1974); for carazolol, Lemoine and Kaumann (1978), Morris et al. (1978)]. With the possible exeption of heterogeneous β-adrenoceptors in sinoatrial nodes of some species (Carlsson et al., 1972; Kaumann et al., 1978), the good agreement of several functional estimates of affinity permit some generalizations on the influence of N-isopropyl and N-t-butyl substituents in determining affinity for myocardial β-adrenoceptors.

Ratios of affinities of N-t-butyl ligands for β-adrenoceptors of rat sinoatrial nodes to those of N-isopropyl ligands were 6.6, 3.0, 3.2 and 1.1 for ligands having 2-chloro-5-methylphenyl, indol, naphthyl and carbazol rings, respectively; ratios obtained with β-adrenoceptors coupled to the adenylyl cyclase of kitten ventricles were 4.8, 3.0, 2.7 and 0.6. Interestingly, the molecular volume of the rings in question is carbazol > naphthyl > indol > 2-chloro-5-methylphenyl. Thus, there appears to exist an inverse correlation between the molecular volume of the ring and the ability of the N-t-butyl substituent to enhance affinity for the myocardial β-adrenoceptor with respect to the N-isopropyl substituent.

N-t-butyl ligands may be more lipophilic than their corresponding N-isopropyl ligands. Enhanced affinity for myocardial β-adrenoceptors of N-t-butyl ligands with small ring volume as compared to their N-isopropyl ligands may be the result of better accomodation with an accessory lipophilic locus of the receptor. On the other hand, the N-t-butyl ligand with a

large ring (i.e. carbazole) may not be able to match better than its N-isopropyl ligand the accessory site, perhaps because of steric hindrance with some β-adrenoceptor component. The contribution to affinity of the N-t-butylamino substituent manifests itself when the affinity of the corresponding N-isopropylamino ligand is relatively low (e.g. KL 1006 vs. bupranolol), but is not apparent when the affinity of the ligand is high (e.g. carazolol vs. tert. butylcarazolol). It appears therefore, that the effect of the tert. butylamino substituent in enhancing affinity is not simply additive with the other molecular components that determine affinity.

As seen previously with pindolol (Barrett and Carter, 1970; Kaumann, 1973; Bilski and Wale, 1976) and carazolol (Lemoine and Kaumann, 1978), these ligands caused positive chronotropic effects. In addition, tert. butylpindolol and tert. butylcarazolol also produced positive chronotropic effects of similar magnitude as their N-isopropyl analogues. Positive chronotropic effects of the 4 ligands were only detected at concentrations substantially higher than those causing β-adrenoceptor blockade. Similar dissociations between stimulation and blockade have been observed in kitten and rat heart with pindolol and other partial agonists (Kaumann, 1973; Bilski and Wale, 1976; Kaumann et al., 1978). The mechanism of the separation between blockade and stimulation is still not clear (Birnbaumer and Kaumann, 1979). However, whatever its explanation, the phenomenon persists when the N-isopropyl group of pindolol and carazolol is replaced by a N-t-butyl group.

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