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The Pharmacological Classification of Practolol and Chloropractolol

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SUMMARY

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The classification of *beta* adrenoceptor subtypes has apparently received substantial confirmation from the cardioselective properties of practolol, so-called $beta_1$ receptor blockade. This interpretation assumes that practolol has no relevant pharmacological properties other than *beta* receptor antagonism; the present report challenges that assumption. Practolol was studied to elucidate the reported alkylating properties of chloropractolol; although no evidence of alkylation was found, the results with chloropractolol are included as a check on the self-consistency of the pharmacological analyses done on rat isolated atria and coronary-perfused hearts. Practolol and chloropractolol had, respectively, 0.3 and 0.4 times the agonist activity of isoprenaline on atrial pacemaker frequency. Using the method of Barlow, Scott, and Stephenson [(1967) *Br. J. Pharmacol.*, **31**, 188-196], apparent equilibrium dissociation constants (pK_P) for these partial agonists were calculated to be 6.8 and 7.8, respectively. However, attempts to estimate the apparent pK_P values directly from Schild plots were frustrated by nonlinearity at low dose ratios. The anomalously low level of antagonism seen at low concentrations appeared to be due to concurrent sensitization of the tissue to isoprenaline, which, decaying more slowly than receptor blockade, became manifest after prolonged washing. The degree of sensitization, namely, a dose ratio of 0.5, was equal to that produced maximally by inhibition of extraneuronal uptake of isoprenaline, using 17β-estradiol, and/or inhibition of catechol *O*-methyltransferase, using U-0521. Neither practolol nor chloropractolol blocked extraneuronal accumulation of isoprenaline measured in perfused hearts, but both depressed the steady-state efflux of 3-methoxyisoprenaline, presumed to be due to catechol *O*-methyltransferase inhibition. Atria maximally sensitized by U-0521 treatment gave Schild plots for practolol and chloropractolol that were linear, had a slope of unity, and gave apparent pK_P values that agreed with values from studies of agonist activities and those calculated independently by a third method, that of Stephenson [(1956) *Br. J. Pharmacol. Chemother.*, **11**, 379-393]. These results are consistent with the predictions of Furchgott's [(1972) in *Handbuch der experimentellen Pharmakologie*, Vol. 33, *Catecholamines* (Blaschko, H. & Muscholl, E., eds.), pp. 203-212, Springer, Berlin] mathematical model for stimultaneous blockade of receptors and agonist uptake, provided that the catechol *O*-methyltransferase in rat atria is inhibited at slightly lower concentrations than the *beta* receptors. The relevance of this finding, if any, to the cardioselective properties of practolol will now need to be examined.

INTRODUCTION

Practolol has been classified as a selective, competitive antagonist of cardiac *beta* adrenoceptors (1). Chloropractolol, the chloroacetyl analogue of practolol, has been proposed as an alkylating agent for cardiac *beta* adrenoceptors (2). Although we could

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not confirm this proposal (3), we did find that chloropractolol and practolol had other actions on our measuring system, namely, rat isolated atria. These differences and their implication for the classification of *beta* adrenoceptor antagonists are the subject of this report.

METHODS

Rat isolated atria. Male hooded rats (250–350 g) were killed by a blow on the head. The right atria were rapidly excised and suspended in 30-ml organ baths containing a solution of Na^+, 170.8 mM; Cl^-, 153.4 mM; K^+, 6.3 mM; Ca^{2+}, 1.3 mM; $H_2PO_4^-$, 1.3 mM; HCO_3^-, 25 mM; and glucose, 25 mM, gassed with 95% O_2–5% CO_2, at 34°, pH 7.2. Rates (per minute) of the spontaneously beating atria were displayed on a potentiometric chart recorder connected to a rate meter (ADG Instruments) that instantaneously measured the reciprocal of the intervals between atrial isometric contractions. The tissues were first exposed to isoprenaline (usually 1 nM) for 10 min and then washed repeatedly for 30–40 min, until a basal rate was achieved that did not change with further washings. All dose-response curves to agonists were obtained by a cumulative method using a common ratio, for increasing doses, of 5 (4). After completion of the control dose-response curve to isoprenaline, the tissues were washed repeatedly until basal atrial rates (within ±5%) were obtained again before antagonists were added. Tissues were equilibrated with antagonists for 45–60 min, changes in basal atrial rate were noted, and then cumulative dose-response curves to isoprenaline were repeated. The location parameter for each family of replicated curves, i.e., the concentration of agonist giving a half-maximal response, was obtained after averaging the concentrations giving equal responses (5).

Measurement of drug antagonism. Since both chloropractolol and practolol showed intrinsic activity on rat atria, the measurement of isoprenaline antagonism was no longer straightforward. Van Rossum (4) has suggested that the displacement of the half-maximal parameters of the dose-response curves be considered as the agonist dose ratio (r) produced by the partial agonist. If the regression of log $(r - 1)$ on log $[P]$ (the molar partial agonist concentration) is linear with a slope not significantly different from unity, it could be considered a graphical representation of the Schild equation (6) for a bimolecular reaction (i.e., when $n = 1$):

$$\log (r - 1) = n \log [P] - \log K_P$$

Under equilibrium conditions, the value of the abscissa at the ordinate intercept [log $(r - 1) = 0$] of a linear regression with a slope of unity has been assumed to be an estimate of log K_P, where K_P is the equilibrium dissociation constant of the partial agonist for the receptor and pK_P is the negative logarithm. If Van Rossum's (4) assumptions that response and stimulus are linearly related and that half-maximal response is the result of half-maximal receptor occupancy are valid for this measuring system, the calculated K_P will be an accurate measure of the equilibrium dissociation constant of the partial agonist and the receptor. However, if these assumptions are not valid, the calculated K_P will be multiplied by a factor involving the efficacy of the partial agonist, the magnitude of which depends upon the relationship between stimulus and response. We have used Van Rossum's method for the calculation of isoprenaline dose ratios but feel that since the relationship between stimulus and response is not known, it would be prudent to consider the calculated K_P values as *apparent* equilibrium dissociation constants of the partial agonist for the receptor, subject to the relative efficacies of isoprenaline and the partial agonist.

Statistical analyses. The sets of dose-response curves to isoprenaline obtained in the presence of chloropractolol and practolol produced replicate estimates (arrays of ordinates) of log $(r - 1)$ for each concentration of antagonist. An estimate of how well the array means conformed to a linear regression (i.e., Schild plot) was gained by comparing the magnitude of the deviations of array means from the regression line with the within-array variation (7). This test for linearity yielded a variance ratio (F), which in turn indicated whether or not

the deviations of the array means were statistically different from the computed regression line. Where Schild regressions had slopes not significantly different from each other, an analysis of covariance was used to determine whether or not the regressions were significantly different with respect to their positions along the x axis. Such a test compared the difference of ordinate values at a given abscissa with the standard error of this difference to yield a value of t (the null hypothesis being that the lines coincide) (7).

Estimate of equilibrium constants from agonist activity. As chloropractolol and practolol were partial agonists in this preparation, it was theoretically possible to calculate the apparent K_P by an independent method utilizing the agonist activity of these drugs. Barlow, Scott, and Stephenson (8) have described a method whereby the dose-response curve of a partial agonist could be compared with the dose-response curve of a full agonist in the same tissue to yield an estimate of the equilibrium constant of the partial agonist (K_P). However, they assumed that a considerable receptor reserve existed for the full agonist, and therefore the concentrations of full agonist (A) were considerably less than the equilibrium dissociation constant of the full agonist for the receptor (K_A). As it was not known whether or not this assumption was justified in our experimental system, A was not considered to be necessarily less than K_A in the following calculations. Thus the reciprocals of equiactive concentrations of isoprenaline and chloropractolol could be equated by

$$\frac{1}{[A]} = \frac{e_A \cdot K_P}{e_P \cdot K_A} \cdot \frac{1}{[P]} + \left(\frac{e_A - e_P}{e_P K_A}\right) \quad (1)$$

where e_A and e_P refer to the respective efficacies of isoprenaline (A) and the partial agonist (P) and K_P is the equilibrium dissociation constant of the partial agonist. If a regression of $1/[A]$ on $1/[P]$ was linear with a positive slope and ordinate intercept, an estimate of K_P could be obtained (slope ÷ intercept). It should be noted that the resulting value would be the equilibrium dissociation constant of the partial agonist for the receptor, modified by an efficacy term, $(e_A - e_P)/e_A$. When the efficacy of the full agonist is considerably larger than the efficacy of the partial agonist, $(e_A - e_P)/e_A$ approaches unity and an accurate estimate for K_P results. A double-reciprocal plot is known to be an unsatisfactory method of expressing data to obtain kinetic parameters (9). A regression of Eq. 2

$$[A] = \frac{e_P \cdot K_A}{e_A - e_P} - \frac{[A]}{[P]} \cdot \left(\frac{e_A}{e_A - e_P}\right) \cdot K_P \quad (2)$$

holds three advantages over a double-reciprocal plot: (a) the variance of $1/[P]$ is relatively large in these experiments, and under these circumstances plots of the form of Eq. 2 have been shown to be superior (9); (b) a convenient measure of the 95% limits of the K_P (from the limits of the slope) can be made (as opposed to the difficulties involved with dealing with the variance of the ratio slope ÷ intercept); and (c) double-reciprocal plots often give spuriously "good" linear regressions, whereas a plot of Eq. 2 exaggerates errors and gives a more sensitive indication of whether or not the data comply well with the theoretical relationships. The estimate of K_P is modified by an efficacy term (which approaches unity if $e_A \gg e_P$) and thus will be referred to as the apparent K_P.

Estimation of a partial agonist equilibrium constant by Stephenson's method. Stephenson (10) has derived a relationship between equiactive concentrations of a full agonist in the absence $[A_1]$ and presence $[A_2]$ of a partial agonist $[P]$. This method was based on the assumption that there is a receptor reserve for the full agonist. As this was not known to be a property of our measuring system, the calculations were carried out without this assumption. Thus

$$[A_1] = \frac{[A_2]}{1 + (1 - e_P/e_A) \cdot ([P]/K_P)} + \frac{(e_P/e_A) \cdot ([P]/K_P) \cdot K_A}{1 + (1 - e_P/e_A) \cdot (P/K_P)} \quad (3)$$

where K_A and K_P are the equilibrium dissociation constants and e_A and e_P are the efficacies of the full and partial agonist, respectively. The slopes of regressions of the control dose-response curve concentra-

tions of isoprenaline (A) in the presence of various concentrations of partial agonist provided estimates of the K_P for the partial agonist, where

$$K_P\left(\frac{e_A}{e_A - e_P}\right) = \frac{[P] \cdot \text{slope}}{1 - \text{slope}}$$

The value calculated from a regression of Eq. 3 is the equilibrium dissociation constant of the partial agonist, modified by an efficacy term that approaches unity if $e_A \gg e_P$. As the relative values of the efficacies are not known, the constant obtained will be referred to as the apparent K_P.

Blockade of extraneuronal uptake of isoprenaline. After determination of control responses to isoprenaline, some atria were equilibrated with 17β-estradiol, 5 μM, an inhibitor of extraneuronal uptake of catecholamines (11). After 30 min a second dose-response curve was determined in the presence of the 17β-estradiol.

Metabolism of isoprenaline by catechol O-methyltransferase. Some atria were equilibrated for 30 min with U-0521, 10 nM, a catechol derivative known to inhibit catechol *O*-methyltransferase in this preparation (12). This was the lowest concentration of U-0521 capable of producing sensitization to isoprenaline, and higher concentrations produced no further increase in the magnitude of the sensitization. To avoid oxidation, 0.1 mM ascorbate and 0.1 mM EDTA were added to the medium in the usual way. A second dose-response curve was then determined in the presence of U-0521.

Detection of metabolites of isoprenaline during perfusion of isolated rat hearts. The detection of metabolites was facilitated by using whole hearts perfused by the Langendorff technique (13) with a modified Tyrode solution containing Na^+, 149.2 mM; K^+, 2.7 mM; Ca^{2+}, 1.275 mM; Mg^{2+}, 2.1 mM; Cl^-, 145.3 mM; $H_2PO_4^-$, 0.4 mM; HCO_3^-, 11.9 mM; glucose, 5.0 mM; ascorbic acid, 0.284 mM; and EDTA, 0.04 mM (14). This solution was constantly bubbled with 98% O_2–5% CO_2, heated to 36°, and pumped through the coronary arteries by a peristaltic pump at a constant rate of 8.5 ml/min. The hearts were perfused for periods of 30 min before being exposed to ^{3}H-labeled isoprenaline.

Collection of measurement of 3-methoxyisoprenaline. The isoprenaline metabolite produced by catechol *O*-methyltransferase, 3-methoxyisoprenaline, was measured after a stabilization period in hearts perfused with a solution containing labeled (10 nM) and unlabeled (1 μM) *dl*-isoprenaline. Bönisch and Trendelenburg (14) had previously shown that the rate of efflux of 3-methoxyisoprenaline was constant after 9–10 min of perfusion, and therefore samples of venous outflow were collected for 30 sec after 12 and 16 min, weighed to check the rate of perfusion, and subjected to the following extraction procedure, based on the preferential adsorption of the catechol group, for 3-methoxyisoprenaline. First, 1 ml of the medium was immediately acidified with 0.5 ml of a mixture of 0.5 *N* HCl, 1% EDTA, and 1% sodium metabisulfite (1:1:3). To this were added 1 ml of Tris buffer (2.5 M, pH 8.5) and 0.5 g of chromatography standard, neutral grade Al_2O_3. This mixture was shaken at room temperature for 10 min and centrifuged at 2500 rpm for 5 min. An aliquot of 1 ml of the supernatant was mixed with scintillation fluid and counted in a Packard Tri-Carb 3380 liquid scintillation counter. Automatic external standardization was used to estimate quenching. The efficiency of counting was 20–26%.

After 17 min of perfusion the hearts were cut into four sections, dried by blotting, and weighed. They were then dissolved in Soluene-350 (Packard) for 24–72 hr and counted as above. Two separate samples of arterial perfusion fluid, obtained after removal of the heart, were subjected to the above extraction procedure for 3-methoxyisoprenaline. The radioactivity in the supernatant was a measure of the contamination of the venous samples by nonadsorbed isoprenaline. The venous samples were corrected accordingly. If the venous samples at 12 and 16 min or the two arterial samples differed by more than 5%, the results were discarded. All hearts were beating rhythmically throughout the experiment.

Drugs. Drugs were obtained from the following sources: *dl*-[7-^{3}H]isoprenaline hy-

drochloride (8.4 Ci/mmole), Radiochemical Centre; *dl*-isoprenaline sulfate, *l*-isoprenaline hydrochloride, and 17β-estradiol, Sigma Chemical Company; *dl*-practolol, a generous gift from Imperial Chemical Industries; reserpine, Ciba; L-ascorbic acid and EDTA, BDH Chemicals; and U-0521 (3′,4′-dihydroxy-2-methylpropiophenone), a generous gift from the Upjohn Company.

Isoprenaline and U-0521 were dissolved in 0.1 mM ascorbate, and all drugs were kept out of direct light and on ice. Chloropractolol, synthesized by Dr. K. Bowden, was dissolved in distilled water immediately before use.

RESULTS

Agonist properties of chloropractolol. Chloropractolol produced a dose-dependent tachycardia that could be inhibited by propranolol. A Schild plot for propranolol with chloropractolol as the agonist had a slope not significantly different from unity (0.9; 95% limits, 0.5–1.4) and yielded a pK_P for propranolol of 8.9 (8.4–10.3). The agonist response began immediately after the chloropractolol was added and reached a plateau in 10–16 min. The average dose-response curve is shown in Fig. 1A. The agonist activity was not reduced by using atria from rats treated with reserpine, indicating that this activity was not due to release of neurogenic noradrenaline.

The offset rate constant for chloropractolol antagonism at *beta* adrenoceptors was reported recently (3). The rate of offset of the agonist action has now been determined for comparison by exposing tissues to various concentrations of chloropractolol for 30 min, washing the atria at 5-min intervals, and then noting the decay of the drug-induced tachycardia. The steady-state decay was adequately described by a linear semilogarithmic regression of the tachycardia (expressed as a percentage of the initial agonist response, using a log scale) on time in minutes. The rate constant for the offset of tachycardia was obtained from the slope of this linear regression ($k_{off} = 0.693/t_{1/2}$) (15).

The rate constants for the decay of tachycardia were not independent of the concentration of chloropractolol (Fig. 2A), and a regression of log k_{off} on the log of the concentration appeared to be linear (Fig. 2B). This regression was not significantly different from a regression of the log k_{off} for antagonism (of isoprenaline responses) by chloropractolol (3) on the log of the concentration of antagonist (analysis of covariance of lines; $t = 1.4$, d.f. = 12). This showed that the dependence of the rate of offset of chloropractolol activity on concentration, as measured by either the agonist or antagonist activity, was the same.

Dose-response curves of the agonist activity of isoprenaline and chloropractolol were compared by the method of Barlow, Scott, and Stephenson (8) (see METHODS) to estimate the equilibrium dissociation constant of chloropractolol for *beta* adrenoceptors.

Data from a comparison of the dose-response curves to chloropractolol with the mean dose-response curve to isoprenaline in rat atria [where all known removal processes for isoprenaline, namely, chemical oxidation (4 μM EDTA), uptake into cardiac muscle (5 μM 17β-estradiol), and methylation by catechol *O*-methyltransferase (10 nM U-0521), had been inhibited] provided the linear regression shown in Fig. 1B. The estimate of the apparent pK_P for chloropractolol from the agonist response was 7.8 (95% limits, 7.3–8.0).

Agonist properties of practolol. An agonist dose-response curve to practolol could also be obtained (Fig. 1). A similar positive chronotropic effect in anesthetized rats has been reported for this compound (16). Propranolol satisfied the conditions for simple competitive antagonism of practolol-induced tachycardia. The Schild plot for propranolol, with practolol as the agonist, had a slope of 0.9 (95% limits, 0.6–1.2) and yielded an apparent pK_P for propranolol of 9.0 (8.6–9.9). The decay of the practolol-induced tachycardia with washing was not independent of concentration, and the rate of offset (k_{off}) showed a linear correlation with log concentration similar to that seen for chloropractolol (slope = −0.6, with 95% confidence limits, −0.4 to −0.8; intercept = −5.2 to −5.5). This linear regression was not significantly different from the regres-

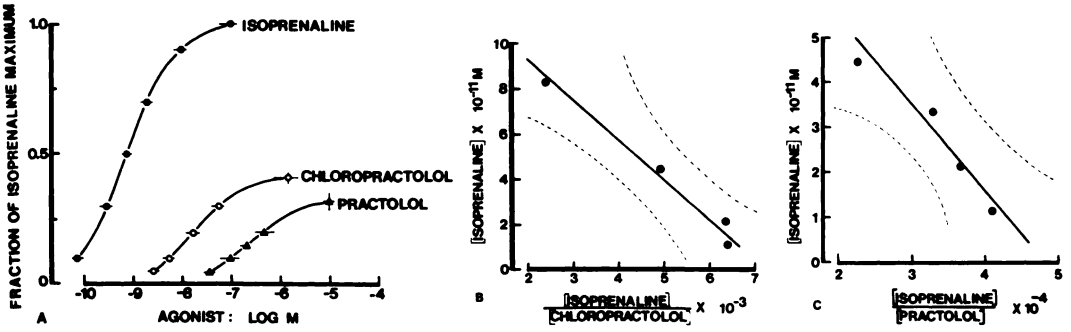

FIG. 1.

A. Agonist responses to isoprenaline, chloropractolol, and practolol. Ordinate: increase in atrial frequency expressed as a fraction of the maximal response to isoprenaline in the same preparation. Abscissa: molar concentrations of agonist (log scale). The means of the concentrations of agonist producing 0.1, 0.3, 0.5, 0.7, and 0.9 times the maximal isoprenaline response were calculated to give the mean dose-response curve. The bars represent the standard errors of the means of these concentrations. ●, mean dose-response curves to isoprenaline (n = 15). The mean concentration of isoprenaline producing half the maximal response was 0.75 nM (95% limits, 0.45–1.04). From a much larger sample of rat atria (n = 92) with a mean basal atrial rate of 283 min^{-1} (277–289), isoprenaline produced a mean maximal increase in rate of 157 min^{-1} (152–162). ○, mean dose-response curve to chloropractolol. The concentration of chloropractolol producing half its own maximal response was 16 nM (95% limits, 10–20). Chloropractolol produced a maximal respons e 0.4 (0.36–0.46) times that of isoprenaline (n = 13). △, mean dose-response curve to practolol. The concentration of practolol producing half its own maximal response was 190 nM (95% limits, 120–260). The mean maxi m al response to practolol was 0.32 (0.26–0.38) times that of isoprenaline.

B. Estimation of the equilibrium constant for chloropractolol. Regression of the concentrations of isoprenaline producing 0.05, 0.1, 0.2, and 0.3 times the maximal response to isoprenaline (ordinate) on these same concentrations divided by equiactive concentrations of chloropractolol (abscissa). The regression is linear (F = 0.54, n_1 = 2, n_2 = 48) and provides an estimate of the apparent K_P for chloropractolol. The slope is 1.6×10^{-8} (95% limits, 0.93–2.5), giving an estimate for the pK_P of 7.8 (7.45–8.03). The dashed lines indicate the 95% confidence limits of the regression.

C. Estimate of the equilibrium constant for practolol. Regression of the concentrations of isoprenaline producing 0.05, 0.1, 0.15, and 0.2 times the maximal response to isoprenaline (ordinate) on these same concentrations divided by equiactive concentrations of practolol (abscissa). The regression is linear (F = 0.25, n_1 = 2, n_2 = 40) and has a slope of 1.80×10^{-7} M (0.6–8). The estimate of the apparent pK_P for practolol from this regression is 6.8 (6.1–7.2). The dashed lines indicate the 95% confidence limits of this slope.

sion of log k_{off}, for the antagonism of isoprenaline responses, on log concentration of practolol previously reported (analysis of covariance of lines; t = 2.25, d.f. = 6) (3).

As with chloropractolol, the agonist dose-response curves to practolol were also used to calculate an estimate of the apparent pK_P for this partial agonist by the method of Barlow, Scott, and Stephenson (8). The linear regression is shown in Fig. 1C. The estimate of the pK_P for practolol from this regression was 6.8 (95% limits, 6.4–7.2).

Antagonist properties of chloropractolol. Chloropractolol antagonized the atrial rate responses to isoprenaline (Fig. 3A), and the maximal responses to isoprenaline also increased significantly (paired t-test; t = 7.8, d.f. = 24, $p < 0.001$). However, a significant increase in the maximal responses to isoprenaline was also seen in parallel control atria without chloropractolol but only washed for 90 min (paired t-test; t = 4.0, d.f. = 7, $p < 0.01$; see also Fig. 5B). There was no significant difference between the increases in the control preparations and those treated with chloropractolol and washing (t = 1.7, d.f. = 31). The dextrad displacements of the half-maximal location parameters of the isoprenaline dose-response curves shown in Fig. 3A provided data for the Schild plot shown in Fig. 3B. Note that 10 nM chloropractolol produced an anomalously low degree of antagonism, leading to nonlinearity in the Schild plot, in spite of the fact that this concentration produced a significant agonist response

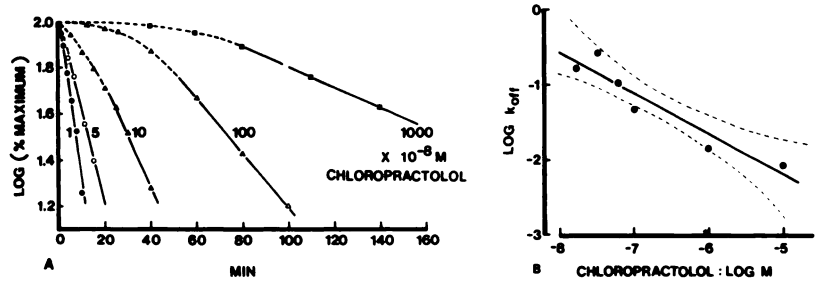

FIG. 2. *Agonist activity of chloropractolol*

A. Decay of chloropractolol-induced tachycardia with washing. Ordinate: tachycardia, expressed as a percentage of the maximal tachycardia obtained with that concentration of chloropractolol (log scale). Abscissa: time (minutes) of washing after equilibration of atria with chloropractolol for 30 min. The apparent first-order elimination rate constant (k_{off}) for chloropractolol was calculated from the linear portions (solid lines) of the decay curves. While the elimination of chloropractolol from the receptor compartment appeared saturable at higher concentrations (as shown by the change in slope of the decay curve), the decay curves were linear thereafter and a k_{off} could be estimated (15).

B. Regression of the rate of decay of tachycardia on concentration of chloropractolol. Ordinate: rate of offset of tachycardia (log scale). Broken lines represent the 95% confidence limits of the slope of the best least-squares fit of the solid line to the data points. Slope of the regression = −0.53 (95% confidence limits, −0.33 to −0.73); intercept = 4.8 (4.3–5.3).

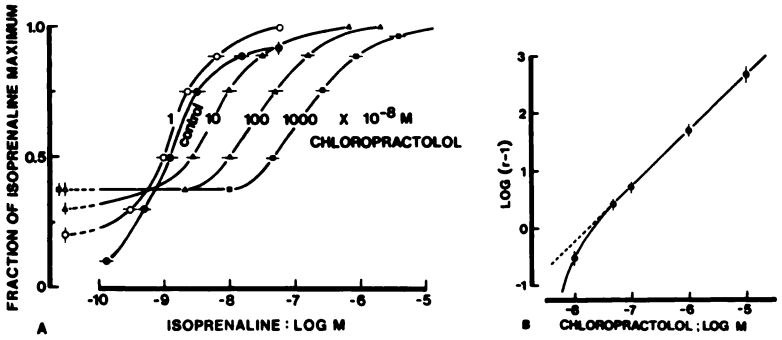

FIG. 3. *Antagonism by chloropractolol of responses to isoprenaline in normal atria*

A. Mean dose-response curves to isoprenaline. Ordinate: increase in atrial frequency produced by isoprenaline, expressed as a fraction of the maximal response to isoprenaline. Abscissa: molar concentrations of isoprenaline (log scale). Multiples of 10^{-8} M refer to the molar concentration of chloropractolol ("control" indicates the control response to isoprenaline before chloropractolol). Ordinate values to the left of the x axis represent atrial responses to chloropractolol alone (basal rates). Atria were equilibrated with chloropractolol for not less than 45 min. Bars represent standard errors of the means.

B. Schild plot for isoprenaline-chloropractolol interaction in rat atria. Ordinate: $(r - 1)$, where r is the dose ratio of concentrations of isoprenaline producing half the maximal response (log scale). Abscissa: concentrations of chloropractolol (log scale). Dose ratios were obtained from the dose-response curves shown in Fig. 3A (n = 34). The regression is nonlinear ($F = 3.09$, $n_1 = 29$, $p < 0.05$) and cannot be used to estimate the K_P. The portion of the regression corresponding to dose ratios greater than 3 (ordinate > 0.3) on the abscissa is linear ($F = 1.27$, $n_1 = 2$, $n_2 = 21$) with a slope of 1.0 (0.9–1.1). Bars represent standard errors of the means.

(Fig. 3A). Associated with this, the family of dose-response curves did not show the common crossover point expected from the uncomplicated interaction between an agonist and a partial agonist. The nonlinearity of the Schild regression appeared at dose ratios of less than 3, a region where accurate measurements were most difficult to obtain. However, if the common practice of using only dose ratios greater than 5 for Schild regressions had been followed, the regression for chloropractolol would have

been linear with a slope not significantly different from unity. Such a plot would have yielded an apparent pK_P of 7.5 (95% limits, 7.4–7.6). The dose ratio for 10 nM chloropractolol calculated by extrapolation of this linear regression of 1.46 (95% limits, 1.3–1.7) was significantly different from the dose ratio of 1.24 found experimentally (95% limits, 1.2–1.3).

Thus the nonlinearity of the Schild plot indicated that something more than simple reversible competitive antagonism was involved.

Antagonist properties of practolol. Practolol produced dextrad displacement of the dose-response curves to isoprenaline (Fig. 4A), generating the Schild plot shown in Fig. 4B. As with chloropractolol, low concentrations of practolol (0.1 μM) sufficient to show significant agonist activity failed to achieve the expected degree of antagonism, so that the Schild regression was not linear. Again, if only dose ratios greater than 5 had been measured, a linear regression with a slope not significantly different from unity would have been found. This linear Schild plot would have given a pK_P for practolol of 6.9 (6.6–7.2). As with chloropractolol, the actual dose ratio produced by 0.1 μM practolol in six atria (1.25; 95% limits, 1.1–1.3) was significantly lower than that predicted by the linear portion of the Schild plot (2.0; 95% limits, 1.4–3.0). The pronounced curve in the Schild plot again indicated that practolol had another effect on this tissue, which became apparent at low concentrations.

Chloropractolol-induced sensitization to isoprenaline. The nonlinearity of the Schild plot for chloropractolol occurred at 10 nM (Fig. 3B), and so the effects of this concentration were investigated in more detail.

After equilibration of seven atria with 10 nM chloropractolol for 45 min and subsequent determination of the response to isoprenaline (total time, 60 min), the mean dose ratio for isoprenaline was 1.24 (95% limits, 1.2–1.3). The atria were then washed for 30 min, and the responses to isoprenaline were redetermined. The dose-response curve was found to have shifted to the left. The responses to isoprenaline of the seven atria before, during, and after equilibration with 10 nM chloropractolol are shown in Fig. 5A. The sensitization to isoprenaline was not observed in parallel control atria washed for 90 min (Fig. 5B).

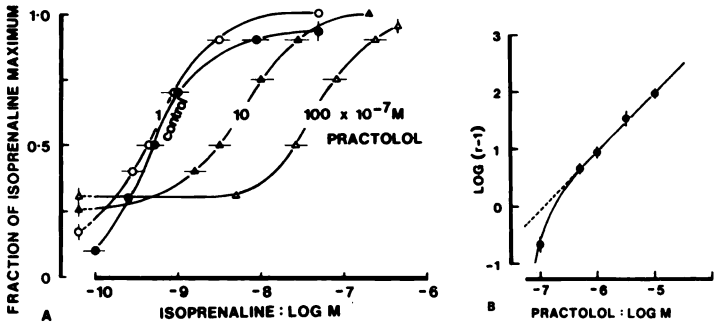

FIG. 4. *Antagonism by practolol of responses to isoprenaline*

A. Mean dose-response curves to isoprenaline. Ordinate: increase in atrial frequency produced by isoprenaline, expressed as a fraction of the maximal response to isoprenaline. Abscissa: concentrations of isoprenaline (log scale). Multiples of 10^{-7} M refer to the molar concentration of practolol ("control" indicates the control response to isoprenaline before practolol). Ordinate values to the left of the x axis represent atrial responses to practolol alone (basal rates). Atria were equilibrated with practolol for no less than 45 min. Bars represent standard errors of the means.

B. Schild plot for isoprenaline-practolol interaction in rat atria. Ordinate: $(r - 1)$, where r is the dose ratio of concentrations of isoprenaline producing half the maximal response to isoprenaline (log scale). Abscissa: concentrations of practolol (log scale). Dose ratios were obtained from the dose-response curves shown in Fig. 4A ($n = 28$). The regression is nonlinear ($F = 3.17$, $n_1 = 3$, $n_2 = 23$, $p < 0.05$) and cannot be used to estimate the K_P. The portion of the regression corresponding to dose ratios greater than 5 (ordinate > 0.6) on the abscissa is linear ($F = 0.35$, $n_1 = 2$, $n_2 = 18$) with a slope of 1.0 (0.8–1.1). Bars represent standard errors of the means.

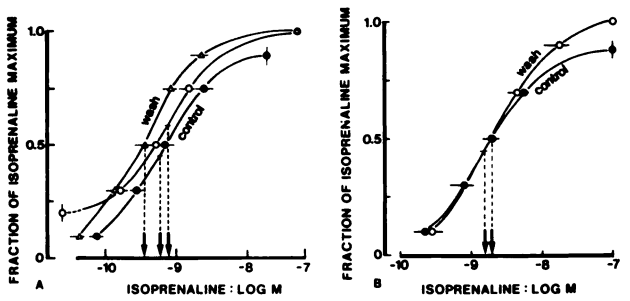

FIG. 5. *Sensitization to isoprenaline after chloropractolol; mean dose-response curves to isoprenaline*
Ordinates: increase in atrial frequency produced by isoprenaline, expressed as a fraction of the maximal response to isoprenaline. Abscissae: concentrations of isoprenaline (log scale). Bars represent standard errors of the means. A. ●, control responses before equilibration with chloropractolol; ○, responses to isoprenaline in the presence of chloropractolol (10 nM) equilibrated for 45 min; △, responses to isoprenaline in the same atria after washing for 30 min (n = 9). Arrows indicate the position on the axis of the half-maximal response parameters of the dose-response curves. Mean dose ratio for isoprenaline after equilibration with chloropractolol and washing = 0.5 (95% limits, 0.4–0.6). B. ●, control responses to isoprenaline; ○, responses to isoprenaline in the same atria after 90 min of washing (n = 12). Mean dose ratio for isoprenaline after 90 min of washing = 1.2 (95% limits, 1–1.3).

Subsequent experiments showed that the sensitization to isoprenaline could be produced by a wide range of concentrations of chloropractolol (10 nM–10 μM), provided that the atria were washed for a period sufficient for removal of the *beta* adrenoceptor antagonism. Thus, whereas sensitization to isoprenaline could be observed after a 30-min equilibration of the atria with 10 nM chloropractolol, followed by 30 min of washing, tissues equilibrated for 30 min with 1 μM chloropractolol required a 90-min wash period [in accordance (3) with the dependence of the rate of offset antagonism on concentration] before sensitization to isoprenaline could be observed.

After washout of the isoprenaline antagonism, the sensitization to isoprenaline was present for periods of up to 3 hr. An estimate of the time course for this sensitization was obtained by equilibrating atria with chloropractolol (50 nM) and recording the dose ratios for isoprenaline (as compared with the first, nonsensitized, control dose-response curve) over a wash period of 3 hr. Figure 6 shows that the antagonism of isoprenaline was removed by washing within the first 20 min and that sensitization (expressed as dose ratios for isoprenaline of less than 1.0) persisted for over 3 hr. Parallel control atria showed that the sensitivity to isoprenaline was slightly decreased by washing in these tissues, but this decrease was not statistically significant (paired t-test; t = 0.6, d.f. = 5).

Thus a specific chloropractolol-induced sensitization of rat atria to isoprenaline was revealed, which had a time course different from that which governed chloropractolol-adrenoceptor-mediated events, namely, tachycardia and isoprenaline antagonism. A new, stable, control dose-response curve to isoprenaline could be obtained in atria that had been equilibrated with chloropractolol and washed until all adrenoceptor antagonism by this drug had been removed. The atria were more sensitive to isoprenaline, and thus the control response to isoprenaline will be referred to as the sensitized control response. Comparison of atrial responses obtained in the presence of chloropractolol (10 nM) with the sensitized control responses produced a mean dose ratio for isoprenaline of 2.0 (1.8–2.3). Surprisingly, this dose ratio was significantly larger than the one already predicted from the Schild equation using nonsensitized control data (this dose ratio was 1.46; 95% limits, 1.3–1.7). This result suggested that atria that had been treated with chloropractolol and therefore displayed a sensitized response to isoprenaline belonged to a different population, in the statistical sense, from normal atria. This sensitization, which served to produce underestimates of the dose ratios, was demonstrable over a wide

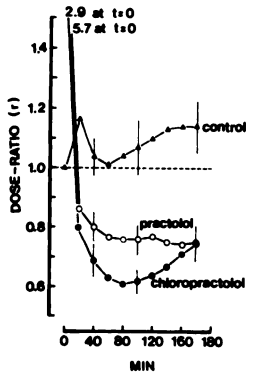

FIG. 6. *Time course for sensitization of atria to isoprenaline after chloropractolol and practolol*

Ordinate: dose ratios for half-maximal concentrations of isoprenaline. Abscissa: time (minutes) of washing after incubation of atria with antagonist for 60 min. ▲, control atria not treated with antagonist (n = 6); ●, atria equilibrated with 50 nM chloropractolol for 60 min (n = 5); ○, 0.5 μM practolol (n = 6). Bars represent standard errors of the means.

range of chloropractolol concentrations, thus implying that the dose ratios over this range could be significantly larger when calculated with respect to sensitized controls. The responses to isoprenaline of 19 atria in the presence of a range of concentrations of chloropractolol and the sensitized control responses (obtained after equilibration and washout of chloropractolol) are shown in Fig. 7A. Note that the difference in maxima between control and treated curves is no longer evident and the curves now have a common crossover point. These dose-response curves provided data for another Schild plot, which was linear and now had a slope of 0.9 (0.8–1.0) (Fig. 7B). Thus, after correction for the chloropractolol-induced sensitization of the atria to isoprenaline, chloropractolol appeared to behave as a simple competitive antagonist of *beta* adrenoceptors, with an apparent pK_P of 8.0 (7.9–8.2). An analysis of the covariance of this linear regression and the linear portion of the regression from nonsensitized control data (dose ratios calculated from the control response obtained before equilibration with chloropractolol) showed the lines, and therefore the estimates of the apparent K_P, to be significantly different (see legend to Fig. 7B).

Practolol-induced sensitization to isoprenaline. Sensitization to isoprenaline of the same order of magnitude (dose ratio = 0.6; 95% limits, 0.5–0.7) as observed with chloropractolol was found in five atria equilibrated with 0.1 μM practolol for 45 min and washed for 45 min; this was the concentration that produced the nonlinearity in the Schild plot (see Fig. 4B). The only differences between practolol and chloropractolol with respect to this sensitization were that 10 times the concentration and slightly longer wash periods were required with the former. The sensitization also persisted for over 3 hr (see Fig. 6 for time course).

Sensitization to isoprenaline after blockade of extraneuronal metabolism. Sensitization that produces a parallel shift to the left of a dose-response curve to an agonist is very often the result of inhibition of a previously existing removal process for the agonist in the tissue (17, 18). The sensitization to isoprenaline produced here by both chloropractolol and practolol suggested inhibition of a previously existing removal mechanism for isoprenaline. The most likely candidate for isoprenaline removal is extraneuronal uptake of isoprenaline (uptake 2) or catechol *O*-methyltransferase-catalyzed methylation.

Blockade of the extraneuronal uptake of isoprenaline by 17β-estradiol (11), 5 μM, produced statistically significant sensitization to isoprenaline (Fig. 8A), not different from that produced by either chloropractolol or practolol.

Inhibition of the catechol *O*-methyltransferase-mediated methylation of isoprenaline by U-0521 (12), 10 nM, also produced sensitization of the atria to isoprenaline (Fig. 8B), the magnitude of which was not significantly different from that produced by chloropractolol, practolol, or 17β-estradiol. Wöppel and Trendelenburg (17) have already shown that inhibition of catechol *O*-methyltransferase in rat atria was as effective as inhibition of extraneuronal uptake by phenoxybenzamine in sensitizing this tissue to isoprenaline.

Uptake and metabolism of isoprenaline in whole hearts. These experiments suggested that chloropractolol or practolol interfered with the disposition of isoprenaline

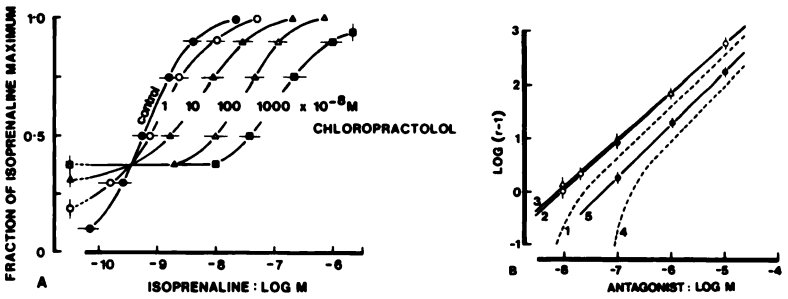

FIG. 7. *Antagonism of isoprenaline by chloropractolol and practolol in sensitized atria*

A. Antagonism of responses to isoprenaline calculated from control dose-response curves obtained after equilibration with chloropractolol and washing; mean dose-response curves to isoprenaline. Ordinate: increase in atrial frequency produced by isoprenaline, expressed as a fraction of the maximal response to isoprenaline. Abscissa: concentrations of isoprenaline (log scale). Multiples of 10^{-8} M refer to the molar concentration of chloropractolol ("control" refers to the control response to isoprenaline obtained in atria previously equilibrated with chloropractolol and washed to remove *beta* adrenoceptor antagonism—the sensitized control response). Ordinate values to the left of the x axis represent atrial responses to chloropractolol alone. Bars represent standard errors of the mean. Atria were equilibrated with antagonist for at least 45 min.

B. Schild plots for chloropractolol and practolol obtained under different experimental conditions. Ordinate: $(r - 1)$, where r is the dose ratio of concentrations of isoprenaline producing half the maximal response (log scale). Abscissa: concentrations of antagonists (log scale). Curve *1*, dose ratios calculated from control responses obtained before equilibration with chloropractolol ($n = 34$). This regression is the same as that shown in Fig. 3B and is included for comparison. It is significantly different (with respect to the abscissa intercepts) from regression *2* ($t = 5.42$, $n = 42$, $p < 0.01$) and regression *3* ($t = 5.67$, d.f. = 33, $p < 0.001$). Curve *2*, dose ratios calculated from dose-response curves of atria equilibrated with chloropractolol and washed for sufficient lengths of time to remove antagonism. These tissues exhibited a sensitized control response to isoprenaline. The regression is linear ($F = 0.26$, $n_1 = 3$, $n_2 = 14$), with a slope of 0.9 (0.8–1.0) ($n = 19$). Analyses of covariance of lines showed this regression to be significantly different (with respect to the abscissa intercepts) from regression *1* (see above) but not from regression *3* ($t = 1.95$, d.f. = 26) ($n = 19$). Curve *3*, antagonism of isoprenaline responses by chloropractolol measured in atria previously equilibrated with U-0521 to inhibit catachol *O*-methyltransferase activity ($n = 8$). This regression is linear ($F = 0.1$, $n_1 = 1$, $n_2 = 5$), has a slope of 0.9 (0.8–1.0), and is significantly different from regression *1* but not regression *2* (see above). Curve *4*, dose ratios calculated from control responses obtained before equilibration with practolol ($n = 28$). This regression is the same as that shown in Fig. 4B and is included for comparison. The regression is significantly different (with respect to the abscissa intercepts) from regression *5* ($t = 3.7$, d.f. = 34). Curve *5*, antagonism of isoprenaline responses by practolol measured in atria previously equilibrated with U-0521 ($n = 8$). This regression is linear ($F = 0.3$, $n_1 = 1$, $n_2 = 5$), with a slope of 1.0 (0.8–1.2). Bars represent standard errors of the means.

in rat atria. Therefore their effect on the uptake and metabolism of isoprenaline was studied. Pilot experiments on isolated rat atria showed that very low levels of 3-methoxyisoprenaline could be recovered, so that this preparation was unsuitable for accurate determinations of uptake and methylation of isoprenaline. From experiments on rat heart ventricle slices and whole hearts, Bönisch and co-workers (18) concluded that the perfused heart (Langendorff preparation) was well suited to the assay of catechol *O*-methyltransferase activity and extraneuronal uptake. The results of uptake experiments of this kind are shown in Fig. 9A.

The relative amounts of isoprenaline and 3-methoxyisoprenaline inside the heart tissue were not determined, and the ordinate values are the total amounts of isoprenaline and metabolite measured after the 17-min perfusion. Chloropractolol, practolol, and U-0521 had no significant effect on the uptake of isoprenaline in whole rat hearts. The effects of these drugs on the initial rate of uptake of isoprenaline were not determined; thus a small effect on initial uptake could not have been seen in these experiments. Significant effects were observed, however, on the steady-state efflux of 3-methoxyisoprenaline, the major metabolite formed from isoprenaline by the action of

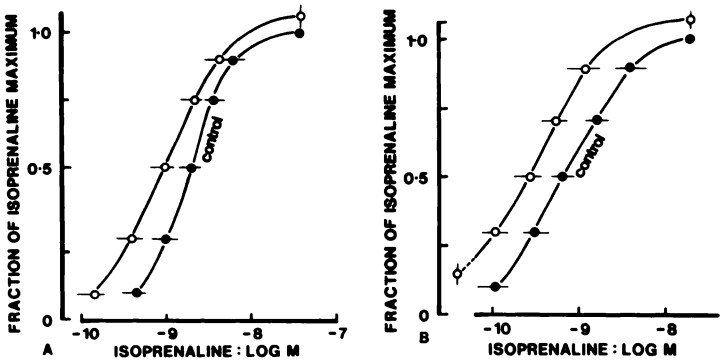

FIG. 8. *Effects of inhibition of removal processes for isoprenaline on atrial responses to isoprenaline*

Ordinates: increases in atrial frequency produced by isoprenaline, expressed as a fraction of the maximal response to isoprenaline obtained before inhibition. Abscissae: concentrations of isoprenaline (log scale). A. ●, control responses to isoprenaline; ○, responses after 17β-estradiol (5 μM) for 45 min (n = 4). Mean dose ratio for isoprenaline concentrations producing half the maximal response = 0.5 (0.3–0.7). B. ●, control responses to isoprenaline; ○, responses after U-0521 (10 nM) for 45 min (n = 8). Mean dose ratio for isoprenaline concentrations producing half the maximal response = 0.6 (0.5–0.8). Bars represent standard errors of the means.

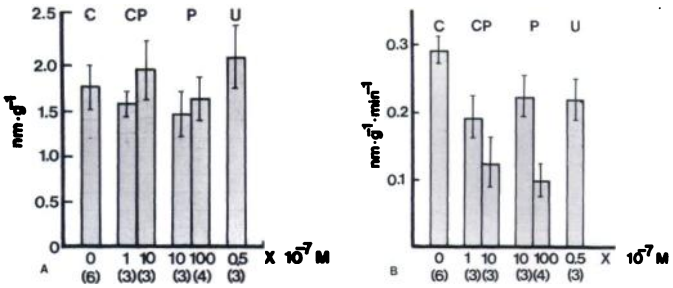

FIG. 9. *Effects of drugs on uptake and O-methylation of isoprenaline in rat hearts*

A. Extraneuronal uptake of isoprenaline into rat hearts. Ordinate: picomoles of isoprenaline plus 3-methoxyisoprenaline per gram stored in cardiac muscle after a 17-min perfusion period. Abscissa: multiples of 10 represent the perfusion concentrations of drugs ($\times 10^{-7}$ M) beneath each bar. Numbers in parentheses represent the number of hearts.

B. Efflux of 3-methoxyisoprenaline from perfused rat hearts. Ordinate: picomoles of 3-methoxyisoprenaline per gram per minute obtained from perfused rat hearts after perfusion with drugs for 30 min. Abscissa: multiples of 10 represent the perfusion concentrations of drugs beneath each bar. Numbers in parentheses represent the number of hearts. C, control; CP, chloropractolol; P, practolol; U, U-0521. Lines represent standard errors of the means.

catechol *O*-methyltransferase. A significant dose-dependent reduction of the rate of efflux of 3-methoxyisoprenaline was produced by both chloropractolol and practolol (see Fig. 9B). Therefore inhibition of catechol *O*-methyltransferase may be the mechanism by which chloropractolol and practolol produce the sensitization to isoprenaline.

Activity of chloropractolol and practolol after inhibition of methylation. To test the hypothesis that the anomalous receptor antagonism and sensitization to isoprenaline produced by these drugs was a result of the inhibition of catechol *O*-methyltransferase-mediated removal process for the agonist, the interaction between chloropractolol and isoprenaline was studied in atria equilibrated with U-0521. The Schild plot was linear, with a slope of unity (Fig. 7B), and yielded an apparent pK_P of 8.1 (7.9–8.4). No further potentiation of isoprenaline responses was observed after washing atria previously equilibrated with chloropracto-

lol and U-0521. Analysis of covariance indicated that this Schild plot was not significantly different from the Schild plot using sensitized controls (Fig. 7B) ($t = 1.95$, $n_1 = 8$, $n_2 = 19$) but was different from the linear portion of the Schild plots from nonsensitized controls ($t = 5.67$, $n_1 = 8$, $n_2 = 25$, $p < 0.001$). Thus consistent values for the apparent K_P of chloropractolol, obtained from linear Schild plots, were achieved in experiments in which the sensitization to isoprenaline was corrected for or if an inhibitor of catechol *O*-methyltranferase was present; a nonlinear Schild plot with a significantly higher apparent K_P was found when neither of these conditions was fulfilled.

An estimate of the apparent K_P for chloropractolol was also obtained by the method of Stephenson (10). Regressions of equiactive concentrations of isoprenaline in the absence and presence of various concentrations of chloropractolol are shown in Fig. 10. The sensitized control response to isoprenaline was taken to be the true control response (dose-response curves shown in Fig. 7A). The same method was used to calculate the apparent K_P for chloropractolol in atria with inhibited catechol *O*-methyltransferase activity. In all these calculations the regressions were linear and gave estimates for the apparent K_P that were comparable to each other and those obtained from either the Schild plots or the method of Barlow, Scott, and Stephenson (8) (quantitative data are given in the legend to Fig. 10).

Similar results were found for practolol. A linear Schild plot with a slope not significantly different from unity was obtained from atria previously equilibrated with U-0521 (Fig. 7B). Both the regression and estimate of the apparent pK_P (7.1; 95% limits, 6.9–7.5) were significantly different from those obtained from atria with uninhibited catechol *O*-methyltransferase. Practolol-induced sensitization to isoprenaline was not observed in atria that had been equilibrated with U-0521. Thus, after the inhibition of catechol *O*-methyltransferase-induced methylation of isoprenaline, practolol behaved as a simple competitive inhibitor of isoprenaline in rat atria.

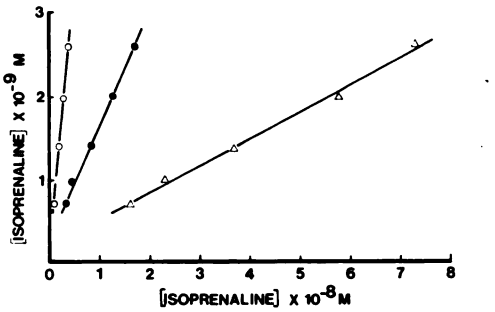

FIG. 10. *Estimation of K_P for chloropractolol by the method of Stephenson (10)*

Ordinate: concentrations of isoprenaline producing agonist responses in atria equilibrated with chloropractolol and washed to remove *beta* adrenoceptor antagonism (sensitized controls). Abscissa: equiactive concentrations of isoprenaline in the presence of various concentrations of chloropractolol: ○, 10 nM [pK_P = 8.0 (7.8–8.1)]; ●, 100 nM [pK_P = 7.6 (7.4–7.7)]; △, = 1 μM [pK_P = 7.7 (7.1–8.0)]. The apparent $K_P = P \cdot [\text{slope}/(1 - \text{slope})]$, where P is the concentration of partial agonist. The apparent $K_P = [e_A/(e_A - e_P)] \cdot K_P$, where K_P is the true equilibrium dissociation constant of the partial agonist for the receptor. In the presence of U-0521 (10 nM), the pK_P estimates from such plots at various concentrations of chloropractolol were: 10 nM, pK_P = 7.8 (7.3–8.0); 100 nM, pK_P = 7.8 (7.7–7.9); 1 μM, pK_P = 7.6 (7.4–7.7); 10 μM, pK_P = 7.7 (7.1–8.0). For practolol they were: 100 nM, pK_P = 7.0 (6.9–7.1); 1 μM, pK_P = 7.2 (7.1–7.3); 10 μM, pK_P = 7.1 (7.0–7.15).

As with chloropractolol, independent estimates for the apparent K_P of practolol were made by the method of Stephenson (10). Linear regressions of equiactive concentrations of isoprenaline in the absence and presence of practolol, in atria with inhibited catechol *O*-methyltransferase activity, were also obtained. The apparent K_P values were consistent with each other and those obtained by other methods (quantitative data are given in the legend to Fig. 10).

Model to represent effect of active removal of agonist. As Furchgott (19) has shown, it is possible to make a model to account for the behavior of the Schild plot for antagonists that also interfere with the removal of agonist. Such calculations are useful to find out whether the observed deviations can be explained by inhibition of catechol *O*-methyltransferase and to define the limiting values of the antagonist equilibrium constant for the removal site.

Furchgott (19) derived an equation relating the concentration of the agonist in the external solution to the concentration in the receptor compartment in the presence of an antagonist:

$$[A_a] = [A_b]\,1 + [B]/K_B \left\{ 1 + \frac{\dfrac{U_m}{k_t \cdot K_{AU}}}{1 + \dfrac{[A_b]\,(1 + [B]/K_B)}{K_{AU}} + \dfrac{[B]}{K_B} \cdot \dfrac{K_B}{K_{BU}}} \right\} \quad (4)$$

where K_B and K_{BU} are the equilibrium constants of the antagonist for the receptor and site of agonist removal, respectively, $[A_a]$ and $[A_b]$ are the respective concentrations of agonist in the external solution and the receptor compartment, k_t is the transfer rate constant for the diffusion of agonist into the receptor compartment, U_m and K_{AU} refer to the maximal rate and equilibrium dissociation constant for the site of agonist removal, respectively, and B is the antagonist.

Utilizing experimental values determined in the present experiments plus those of others, theoretical Schild plots were calculated from Eq. 4 by comparing the value of $[A_b]$ in the absence and presence of the antagonist (compare $[A_a]$ for $[B] = 0$ and for $\log [B]/K_B = -1$ to 3) and multiplying the expected theoretical dose ratio by this factor.

In order to evaluate Eq. 4, a reasonable estimate of $U_m/(k_t \cdot K_{AU})$ was required. Considering the concentrations of isoprenaline used in control dose-response curves (0.1–10 nM) and the K_m for the methylation of isoprenaline in rat hearts of 2.9 μM (14), $A_b \ll K_{AU}$, and it can be shown that (19)

$$\frac{[A_b]}{[A_a]} = \frac{K_{AU}}{K_{AU} + U_m/k_t} \quad (5)$$

The 2-fold potentiation of isoprenaline responses in our preparation after the inhibition of catechol *O*-methyltransferase by U-0521 (see Fig. 8B) indicated that a reasonable value for $[A_b]/[A_a]$ would be 0.5. Thus $U_m/(k_t \cdot K_{AU})$ is approximately equal to 1 for the calculation of theoretical Schild plots for this system.

Equation 4 was calculated for $K_B/K_{BU} = 1$, 2, and 5, and the resulting Schild plots are shown in Fig. 11. If $K_B/K_{BU} = 1$, the experimental Schild plot was parallel to and shifted to the right of the ideal Schild plot by 0.3 log unit—a value corresponding to the magnitude of the maximum sensitization observed when the removal process was inhibited. When $K_B/K_{BU} > 1$, a pronounced bend in the Schild plot occurred at low dose ratios. Thus it can be seen that shifts in the Schild plot occurring at dose ratios of 2 (which would give rise to errors in the estimations of the apparent K_P) resulted in this model system only if the antagonist inhibited the removal process for the agonist at very low concentrations—equal to or less than the K_P. A comparison of the experimental Schild

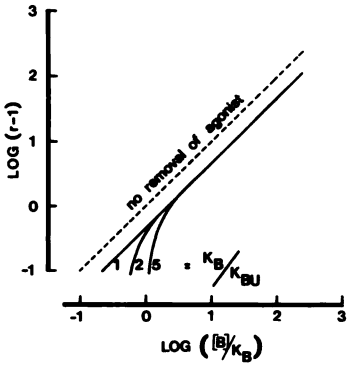

FIG. 11. *Theoretical Schild plots showing effect of removal processes for agonist and inhibition of these processes on the Schild regression*

Ordinate: $(r - 1)$, where r is the dose ratio for concentrations of agonist required to produce half the maximal response to that agonist (log scale). Abscissa: concentration of antagonist as a multiple of the equilibrium constant of the antagonist for the receptor ($[B]/K_B$; log scale). The dashed line indicates the Schild plot obtained when there is no active removal process for the agonist present in the tissue. When an active removal process, possessing parameters characteristic of extraneuronal uptake and metabolism of isoprenaline in rat hearts, is present, the Schild plot will deviate from this ideal relationship. When the antagonist has an appreciable effect on the removal process at low concentrations, Schild plots shifted to the right of the ideal Schild plot will be obtained. The numbers next to these lines refer to the values of K_B/K_{BU}.

plots for chloropractolol and practolol with the theoretical plots suggested that the removal mechanism for isoprenaline could be inhibited by these antagonists in concentrations approximately 0.5 times the apparent K_P.

DISCUSSION

Drugs may be classified by their interactions with cellular macromolecules such as hormone receptors, enzymes, and storage sites. These interactions can be defined by appropriate concentration parameters, usually the locus for half-maximal combination. When drugs react with enzymes, storage sites, or transport processes, changes in the concentration of corresponding substrates or hormones occur that can often lead directly to estimates of a suitable parameter such as the K_I for drug-enzyme interactions. However, drug-receptor interactions present special problems. Although binding constants in drug-receptor interactions can occasionally be measured with minimal ambiguity, indirect estimation based on the analysis of tissue- or enzyme-coupled responses is still the major method. For this method, the relationship (Schild plot) between lateral displacement of dose-response curves, as log $(r - 1)$, and the concentration of antagonist, log $[B]$, provides the minimum necessary (but not necessarily sufficient) conditions. In this test, if the regression is linear over a wide range of antagonist concentrations and has a slope of 1, the system is behaving as though there is simple competitive interaction between agonist and antagonist for a common site. A parameter having the characteristics of a dissociation constant can be calculated from this regression. However, Furchgott (19) has shown, by both theoretical and experimental analysis, that simple competitive antagonism can be associated with nonlinear Schild plots where (a) there is an active tissue removal process for the agonist and (b) the antagonist interferes with the removal process as well as the receptor. Uptake and metabolism often show as much hormone specificity as do the receptor sites, so that it should not be surprising if a specific receptor antagonist also showed some affinity for related removal processes.

Although the antagonist properties of both practolol and chloropractolol were well known, the finding that they had significant agonist properties on the rat isolated atrial preparation posed problems that were both theoretical and practical. The theoretical problem was solved by finding that the agonist activity was not reduced by reserpine, that propranolol had the same pK_B with these as with isoprenaline, and that agonist and antagonist actions decayed at the same rate; the criteria for classifying these agents as partial agonists were satisfied. The practical problem raised by partial agonists is the method of measuring dose ratios. Van Rossum's theoretical treatment, leading to the conclusion that dose ratios should be measured by the shift of the half-maximal concentration parameters, was based on the assumption of a simple linear relationship between occupancy and response (4). Would this assumption be justified in our experiments? We reached an oblique answer by comparing the apparent pK_P values for practolol and chloropractolol obtained from the Schild regressions, using Van Rossum's (4) method, with apparent pK_P values obtained by two independent methods that were based on different assumptions: Barlow, Scott, and Stephenson's (8) and Stephenson's (10). All three of these methods yield K_P values multiplied by an efficacy term, but this term was not the same for all three methods. Experimentally it was found that differences in the estimations of the apparent pK_P values were not larger than the errors in measurement. Therefore we assumed that the efficacy terms modifying the estimates of K_P are not large. We used Van Rossum's method because of the additional information provided by Schild plots.

Using this method, we found that chloropractolol and practolol did not produce simple competitive inhibition of isoprenaline responses, as judged by the nonlinearity of the Schild plot. However, there was another action of these drugs that produced significant sensitization of the atria to isoprenaline and led to an underestimate of the apparent K_P. As outlined by Furchgott (19), certain criteria must be fulfilled before

a valid measurement of the dissociation constant of an antagonist for a receptor can be made. A primary consideration is that the concentration of the agonist in the receptor compartment must stay constant throughout the course of the measurements. If, however, a removal mechanism for the agonist is inhibited during equilibration of the tissue with antagonist, the concentrations of the agonist will be larger than suspected, during the measurement of the antagonism, and the antagonist will appear to be less potent than it really is. The estimates of apparent K_P values for both chloropractolol and practolol were significantly larger when the sensitization to isoprenaline was not corrected for or when the experiments were not done in the presence of U-0521.

The question therefore is: What is the mechanism of this sensitization, and is this process, concurrent with receptor blockade, sufficient to distort the expected relationships for simple competitive antagonism? One possible explanation for the sensitization is inhibition of extraneuronal uptake or removal of the agonist. Bell and Grabsch (20) have shown that extraneuronal uptake is a physiologically important mechanism for the termination of the effects of noradrenaline released from neurons. Inhibition of extraneuronal uptake by 17β-estradiol produced sensitization to isoprenaline in our preparations equal in magnitude to that obtained with chloropractolol or practolol, and a theoretical analysis showed that inhibition of extraneuronal uptake might be sufficient to cause the observed distortion of the Schild plot. However, our experiments, admittedly on perfused whole hearts rather than isolated atria, failed to show inhibition of extraneuronal uptake.

Another possibility is suggested by the work of Wöppel and Trendelenburg (17). They found that U-0521 sensitized rat atria to isoprenaline to the same extent as inhibition of extraneuronal uptake by phenoxybenzamine. Although a high concentration of U-0521 (0.1 mM) was used, they concluded that inhibition of catechol *O*-methyltransferase was the mechanism of sensitization. The magnitude of the maximum sensitization (0.25 log unit) was very similar to that found in our studies with either U-0521 or 17β-estradiol. It has been established subsequently that the K_I for U-0521 inhibiting extraneuronal uptake of isoprenaline into rat heart is high (2.4×10^{-4} M) (18), making it very unlikely that inhibition of isoprenaline uptake is the mechanism of sensitization in our experiments, where the concentration of U-0521 was 10 nM.

Chloropractolol and practolol produced sensitization to isoprenaline equal in magnitude to that obtained with U-0521, and further potentiation with chloropractolol or practolol, after treatment with U-0521, was not observed. These results, in conjunction with the inhibition of the efflux of 3-methoxyisoprenaline, the metabolite of isoprenaline produced by catechol *O*-methyltransferase in rat heart, support the hypothesis that these *beta* adrenoceptor antagonists inhibit this enzyme in rat heart. Buckner, Birnbaum, and O'Conner (21) have also suggested that sotalol inhibits catechol *O*-methyltransferase as well as blocking *beta* adrenoceptors, but their evidence was indirect.

The equilibrium constant for the inhibition of catechol-*O*-methyltransferase would have to be less than or at least equal to the K_p for *beta* adrenoceptor antagonism for the theoretical analysis to be in accord with the experimental Schild plots for chloropractolol and practolol. Concentrations equal to 10 times the K_p were required, however, to cause significant reduction of 3-methoxyisoprenaline efflux. We are not convinced that there is any conflict here. If coronary perfusion of the whole heart is inefficient and if a substrate gradient into cardiac muscle exists for isoprenaline, a theoretical treatment (22) predicts that, as the substrate gradient decreases with enzyme inhibition, a spuriously high value for K_I will be obtained. Furthermore, pilot experiments on a preparation of catechol-*O*-methyltransferase from rat liver showed no inhibition by either chloropractolol or practolol. A similar situation was encountered by Eisenfeld, Iversen, and Axelrod (23), who found that dichloroisoproterenol blocked the *O*-methylation of tritiated catecholamines in perfused, isolated hearts but had no effect on a preparation of catechol

O-methyltransferase isolated from this organ. Isoenzymes of catechol *O*-methyltransferase are known (24), and it is possible that the experiments with chronotropic responses *in vitro* are affected by a relatively small amount of a type of this enzyme unique to the sinoatrial node. Or, more likely, the enzyme may be located in two different compartments, perhaps one associated with membrane-bound enzymes and the other with free intracellular enzymes; a special kind of membrane-bound catechol *O*-methyltransferase has already been described (25, 26).

In conclusion, our results indicate that chloropractolol and practolol may be classified as inhibitors of cardiac catechol *O*-methyltransferase in addition to their action as partial agonists at *beta* adrenoceptors in rat atria. The relevance of this finding, if any, to the cardioselective properties of practolol will now need to be examined.

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