CHARACTERIZATION OF $\beta$-ADRENOCEPTORS IN THE DOG SAPHENOUS VEIN

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Abstract—*In vitro* experiments were carried out on strips of the dog saphenous vein to characterize $\beta$-adrenoceptors mediating relaxation. Four $\beta$-adrenoceptor agonists, isoproterenol, salbutamol, procaterol and $\alpha$-(3,4,5-trimethoxyphenethylaminomethyl)-3,4-dihydroxybenzylalcohol hydrochloride (T-1583), all produced concentration-dependent relaxation of venous strips contracted by $10^{-6}$ M methoxamine. These four drugs behaved as full agonists. In producing venous relaxation, procaterol was about 2.5 times more potent, and salbutamol and T-1583 were 7 and 98 times less potent than isoproterenol, on a molar basis. The concentration-relaxation response curves to the four agonists were shifted in a parallel way to the right by (1-butyl-amino-3-ol-2-propyl)oximino-9 fluorene hydrochloride (IPS 339), a selective $\beta_2$-adrenoceptor antagonist, and by practolol. However, pA$_2$-values for IPS 339 against the four agonists were all nearly 11.0, whereas those for practolol were all nearly 5.7. We conclude that $\beta$-adrenoceptors in the dog saphenous vein mediating relaxation are predominantly of the $\beta_2$ type.

Vascular $\beta$-adrenoceptors were initially classified as $\beta_2$ type by Lands et al. (1, 2) and the classification has been supported by subsequent studies (3, 4). However, concurrent reports have also accumulated suggesting that vascular $\beta$-adrenoceptors may not be homogeneous. Baron et al. (5) have shown in *in vitro* experiments that $\beta$-adrenoceptors of the dog coronary artery resemble the cardiac $\beta$ ($\beta_1$, type) rather than the vascular $\beta$ ($\beta_2$ type) adrenoceptors. Edvinsson and Owman (6) have demonstrated in *in vitro* experiments that $\beta$-adrenoceptors of cat cerebral arteries can be classified as $\beta_1$ type in contrast to $\beta_2$-adrenoceptors in the peripheral vascular bed (with exception of the coronary circulation). Taira et al. (7) have shown in *in vitro* experiments in dogs that $\beta$-adrenoceptors of the renal arterial bed consist of both $\beta_1$ and $\beta_2$ types, whereas the femoral and superior mesenteric arterial beds contain only $\beta_2$-adrenoceptors. With regard to venous $\beta$-adrenoceptors, in *in vitro* experiments Cohen and Wiley (8) found that the rat jugular vein possesses both $\beta_1$- and $\beta_2$-adrenoceptors, although the latter is predominant (9). However, systematic studies of types of $\beta$-adrenoceptors on smooth muscle of veins have apparently not been documented. Since in preliminary experiments we found that the dog saphenous vein was most responsive to various $\alpha$- and $\beta$-adrenoceptor agonists, we quantitatively compared the effects of four $\beta$-adrenoceptor agonists, i.e., isoproterenol, salbutamol, procaterol and T-1583 in producing relaxation of smooth muscle of the dog saphenous vein contracted by methoxamine. Procaterol is a highly selective and potent $\beta_2$-adrenoceptor agonist (10–12).
T-1583 is a selective $\beta_1$-adrenoceptor agonist (13). We also compared the effects of practolol and those of the selective $\beta_2$-adrenoceptor antagonist, IPS 339 (14) in antagonizing relaxation of the dog saphenous vein induced by the four $\beta$-adrenoceptor agonists. Methoxamine, although reported to antagonize tracheal $\beta_2$-adrenoceptors (15), was chosen, since it is classified as a rather selective $\alpha_1$-adrenoceptor agonist (16).

MATERIALS AND METHODS

Mongrel dogs of either sex weighing 6–10 kg were anesthetized with sodium pentobarbital, 30 mg/kg i.v. The lateral saphenous veins were dissected out in a length of about 3–4 cm and freed of connective tissues. Helical strips were cut from the dissected segments in Krebs-Henseleit solution containing 0.057 mM ascorbic acid and 0.027 mM EDTA•2Na. The composition of the solution was as follows: NaCl, 118; KCl, 4.7; CaCl$_2$, 2.55; MgSO$_4$, 1.18; NaHCO$_3$, 24.9; glucose, 11.1 (mM). The helical strips were suspended in organ baths which contained 20 ml of Krebs-Henseleit solution equilibrated with 95% O$_2$ + 5% CO$_2$ and maintained at 37°C. The strips were connected to force transducers (San-ei Instrument, Type 35196), stretched to give 500 mg of resting tension and allowed to equilibrate 1–2 hr before exposure to drugs. Tension developed by smooth muscle of the strips was recorded isometrically on an ink-writing oscillograph (San-ei Instrument, 8S). The dog saphenous vein strips had very little resting tone and were therefore contracted with methoxamine ($10^{-6}$ M). Submaximal (about 55–80% of maximum) contractions induced by $10^{-6}$ M methoxamine were maintainable and reproducible. Once the contraction reached a plateau, $\beta$-adrenoceptor agonists were added, without washing, in a cumulative way to determine concentration-relaxation response curves. The response to each concentration was allowed to level off (more than 5 min) before the succeeding injection was made, and maximum relaxation for each concentration was measured. Relaxation of the contracted strips back to base-line tension represented 100% relaxation. Concentration-relaxation response curves for any of the $\beta$-adrenoceptor agonists used were reproducible in the absence of a given $\beta$-adrenoceptor antagonist, so far as they were determined 4 times. After control concentration-relaxation response curves had been obtained, the strips were washed out repeatedly, and then incubated with appropriate concentrations of practolol or IPS 339 for 1 hr as recommended by Furchgott (17). A second concentration-relaxation response curve for the same $\beta$-adrenoceptor agonist was then determined in the presence of $\beta$-adrenoceptor antagonists. The same procedure was repeated by increasing concentrations of $\beta$-adrenoceptor antagonists, using identical strips. The pD$_2$-value ($-\log$ ED50) was calculated as described by van Rossum (18). $\beta$-Adrenoceptor antagonism was evaluated from pA$_2$ values described by Schild (19) and Arunlakshana and Schild (20).

The drugs used are (±)-methoxamine hydrochloride (Nihonshinyaku), (−)-isoproterenol hydrochloride (Merck), (±)-salbutamol sulfate (Leiras), (±)-procaterol hydrochloride hemihydrate (Otsuka), α-(3,4,5-trimethoxyphenethylaminomethyl)-3,4-dihydroxybenzylalcohol hydrochloride (T-1583) (Tanabe), (±)-practolol hydrochloride (ICI), (±)-(t-butyl-amino-3-ol-2-propyl) oximino-9 fluorene hydrochloride (IPS 339) (kindly provided by Dr. G. Leclerc). All the drugs were dissolved in 0.9% saline.

The concentration-relaxation response curves for $\beta$-adrenoceptor agonists were treated as linear regressions and analyzed for similarities in slope.
RESULTS

Effects of isoproterenol, salbutamol, procaterol and T-1583: Isoproterenol (10^{-10}–10^{-7} M), salbutamol (10^{-9}–3\times10^{-6} M), procaterol (10^{-10}–3\times10^{-8} M) and T-1583 (10^{-8}–10^{-6} M) produced concentration-dependent relaxation of venous strips contracted with 10^{-6} M methoxamine. The four agonists produced maximal relaxation. The concentration-relaxation response curves to the four agonists obtained from five strips for each agonist are shown in Fig. 1. The curves were all parallel. pD_{2}-value was 8.33\pm0.04 for isoproterenol, 7.47\pm0.03 for salbutamol, 8.69\pm0.03 for procaterol and 6.34\pm0.04 for T-1583. Thus, procaterol was about 2.5 times as potent as isoproterenol, whereas salbutamol and T-1583 were about 7 and 98 times less potent than isoproterenol.

Antagonism by IPS 339: IPS 339 in concentrations of 10^{-10}, 3\times10^{-10} and 10^{-9} M produced parallel shifts of the concentration-relaxation response curves for the four \(\beta\)-adrenoceptor agonists to the right with no depression of the maximum response, as shown in Fig. 2. The pA_{2}-value for IPS 339 against isoproterenol was 10.8 and the

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\[ -\log \text{conc}(M) \text{ of agonists} \]

**Fig. 1.** Concentration-relaxation response curves for isoproterenol (○), salbutamol (▲), procaterol (●) and T-1583 (△) obtained in strips of the dog saphenous vein. Each point represents the mean of 5–6 preparations. Vertical bars show S.E.

**Fig. 2.** Upper row: Concentration-relaxation response curves for isoproterenol (a), salbutamol (b), procaterol (c) and T-1583 (d) in the absence (●) and the presence of IPS 339 (○, 10^{-10} M; □, 3\times10^{-10} M; △, 10^{-9} M) obtained in strips of the dog saphenous vein. Vertical bars show S.E. (n=5–6). Lower row: Relation between the log (agonist dose ratio -1) and the negative logarithm of the molar concentration of IPS 339 obtained from the above data.
The slope of the regression line was 0.77. Corresponding values were 10.9 and 0.78 for salbutamol, 11.0 and 0.79 for procaterol, and 10.8 and 0.77 for T-1583.

Antagonism by practolol: Practolol in concentrations of $10^{-6}$, $3 \times 10^{-6}$ and $10^{-5}$ M produced parallel shifts of the concentration-relaxation response curves for the four $\beta$-adrenoceptor agonists to the right with no depression of the maximum response, as shown in Fig. 3. The $\text{pA}_2$-value for practolol against isoproterenol was 5.7 and the slope of the regression line was 0.84. Corresponding values were 5.7 and 0.88 for salbutamol, 5.8 and 0.89 for procaterol, and 5.6 and 0.93 for T-1583.

**DISCUSSION**

In the present study, four $\beta$-adrenoceptor agonists, isoproterenol, salbutamol, procaterol and T-1583 all produced maximal relaxation of smooth muscle of the dog saphenous vein contracted by the relatively selective $\alpha_1$-adrenoceptor agonist, methoxamine. The concentration-relaxation response curves for the four $\beta$-adrenoceptor agonists were all in parallel. In producing relaxation, procaterol was about 2.5 times as potent as isoproterenol, whereas salbutamol and T-1583 were about 7 and 98 times less potent than isoproterenol, on a molar basis. The relative potency of salbutamol to isoproterenol, as determined in the present study is close to the relative potencies obtained in tissues in which $\beta_2$-adrenoceptors are predominant (3, 4, 10–12). The relative potency of procaterol to isoproterenol, as determined in the present study, is rather close to the relative potency observed in the dog tracheobronchus as compared to the dog.
vascular bed (10, 11). Nakajima et al. (13) reported that the potencies of T-1583 in producing positive inotropic, positive chronotropic and hypotensive effects on the open-chest dog are about 1/4, 1/10 and 1/100 those of isoproterenol respectively, and that in relaxing the guinea-pig trachea and the rat uterus T-1583 is about 300 and 100 times less potent than isoproterenol, on a molar basis. Therefore, they classified T-1583 as a selective $\beta_1$-adrenoceptor agonist. The relative potency of T-1583 to isoproterenol determined in the present study is close to the relative potencies obtained by these workers in tissues in which $\beta_2$-adrenoceptors are predominant. Thus, the order of potencies of the four $\beta$-adrenoceptor agonists strongly suggests that $\beta$-adrenoceptors mediating relaxation in the dog saphenous vein are predominantly of the $\beta_2$ type.

The concentration-relaxation response curves for the four $\beta$-adrenoceptor agonists were shifted in a parallel way to the right with application of IPS 339. $pA_2$-values of IPS 339 against the four $\beta$-adrenoceptor agonists were all nearly 11.0. This value is slightly higher than the $pA_2$-value 9.2 for IPS 339 obtained against isoproterenol, by Imbs et al. (14) in the guinea-pig trachea contracted with carbachol. The concentration-relaxation response curves for the four $\beta$-adrenoceptor agonists were also shifted in a parallel way to the right by practolol. However, $pA_2$-values for practolol against the $\beta$-adrenoceptor agonists were all nearly 5.7. This value is consistent with the $pA_2$-values for practolol against isoproterenol as obtained in tissues in which $\beta_2$-adrenoceptors are predominant (14, 21), but differs by slightly greater than 1 from values obtained in tissues in which $\beta_2$-adrenoceptors are predominant (14, 21). Furchgott (22) proposed that to differentiate receptor types the difference in $pA_2$-values should be greater than 0.5. Thus, it appears likely that the antagonism by practolol of the four $\beta$-adrenoceptor agonists, as observed in the present study, involves $\beta_2$-adrenoceptors but not $\beta_1$-adrenoceptors.

The slope of the Schild plot ranged from 0.84 to 0.93 for practolol, indicating that practolol behaves as a competitive antagonist on $\beta_2$-adrenoceptors on smooth muscle of the dog saphenous vein. In contrast, the slopes of the Schild plots for IPS 339 against the four $\beta$-adrenoceptor agonists were all nearly 0.78, being less than unity. However, this does not necessarily mean that IPS 339 does not behave as a competitive antagonist, but instead merely indicates that IPS 339 does not combine with the $\beta_2$-adrenoceptors in the usual bimolecular manner.

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