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EFFECTS OF RING-METHYLATED β-ADRENERGIC BLOCKING AGENTS ON ISOPROTERENOL-INDUCED FREE FATTY ACID MOBILIZATION

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Abstract—The effects of β-adrenergic blocking agents on isoproterenol-induced release of free fatty acids from rat epididymal adipose tissue *in vitro* were studied. The compounds investigated were 1-(3′,4′-dimethyl)phenyl-1-hydroxy-2-isopropylaminoethane (E 3, 4), its 3′-monomethyl (E 3) and 4′- monomethyl (E 4) derivatives, and also 1-(3′,4′-dimethyl)phenoxy-2-hydroxy-3-isopropylaminopropane (P 3, 4) and its 3′-monomethyl (P 3) and 4′-monomethyl (P 4) derivatives.

All drugs of the E series have been shown to act as "dualists with multiple action", i.e. weak agonists with combined competitive and noncompetitive antagonistic effects.

Drugs of the P series showed no significant mimetic effects. These drugs were found to act as "pure" antagonists. The change from the ethanolamine to the oxypropanolamine side-chain led to, (1) disappearance of the intrinsic activity, (2) an increase in the affinity of the specific component, and (3) an increase also in the nonspecific component, noncompetitive antagonism.

Of the drugs studied, P 3 is the most potent adrenergic blocker under our experimental conditions. Irregularities in the calculated pA_2 values related to the drug concentration are discussed.

IN A previous paper[1] we studied the influence of the *N*-substituent in a series of dichlorophenylethanolamine β-adrenergic blocking agents on intrinsic free fatty acid (FFA) mobilizing activity and on antagonistic potency against isoproterenol-induced FFA mobilization. A close relation was found to the known relative affinities of catecholamines for the β-adrenergic receptor in that relative activities of the *N*-substituted dichloro derivatives were: i-Pr > Me > H.

In the present paper an attempt is made to determine the effects of 3′- and 4′-methyl substituents on the ring and of changing the ethanolamine side-chain to a propanediol side-chain, as it is in propranolol.

MATERIALS AND METHODS

Experiments were done *in vitro* on the release of free fatty acids (FFA) from rat epididymal adipose tissue into an albumin-containing Krebs–Ringer phosphate medium. FFA were estimated by Dole's[2] method. The influence of the adrenergic blocking agents in various concentrations on the dose–response curve of FFA mobilizing action of isoproterenol was determined. Each experiment was repeated eight times. The intrinsic activity (α) and the affinity values (pD_2, pA_2 and pD'_2) were estimated according to the method of van Rossum;[3] pD''_2, which represents an orientative value for nonspecific, noncompetitive antagonism, is the negative logarithm of the concentration at which the drug diminishes the effect of the agonist to 50 per cent

solely by the noncompetitive component of its action. Further details of the methods were described previously.[1]

The following drugs were used (Fig. 1): 1-(3',4'-dimethyl)phenyl-1-hydroxy-2-isopropylaminoethane hydrochloride (E 3,4); 1-(4'-monomethyl)phenyl-1- hydroxy-2-isopropylaminoethane hydrochloride (E 4); 1-(3'-monomethyl)phenyl-1-hydroxy-2-isopropylaminoethane hydrochloride (E 3); 1-(3', 4'-dimethyl)phenoxy-2-hydroxy-3-isopropylaminopropane hydrochloride (P 3,4); 1-(4'-monomethyl)phenoxy-2-hydroxy-3-isopropylaminopropane hydrochloride (P 4); 1-(3'-monomethyl)phenoxy-2-hydroxy-3-isopropylaminopropane hydrochloride (P 3); isoproterenol sulfate. All drugs were used as racemates.

The compound designated P 3 is identical with Kö 592 (C. H. Boehringer Sohn).

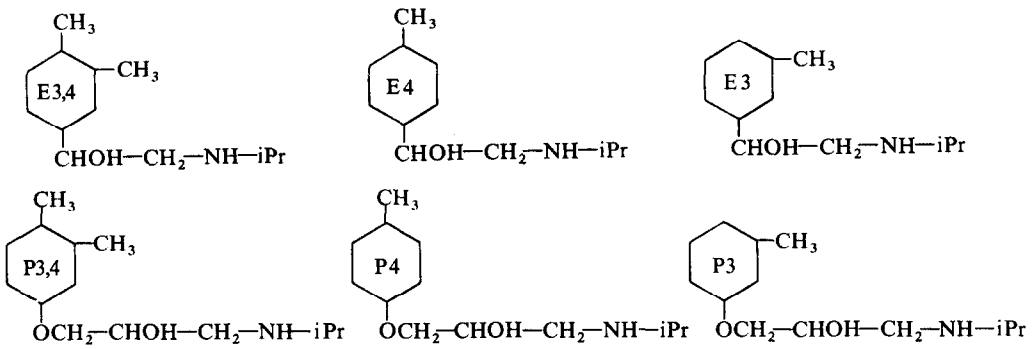

FIG. 1. Structural formulas of the compounds tested. The symbol for each compound is within its ring.

RESULTS

Fig. 2–4 show the effects of compounds of the ethanol (E) series. An agonistic effect is always evident, but it is most pronounced in compound E 3. The parallel shift of the dose–response curves indicates the presence of a component of action characteristic of competitive antagonism; the depression of the maxima indicates a simultaneous noncompetitive interaction.

Figs 5–7 present the data for the drugs of the propanediol (P) series. It should be

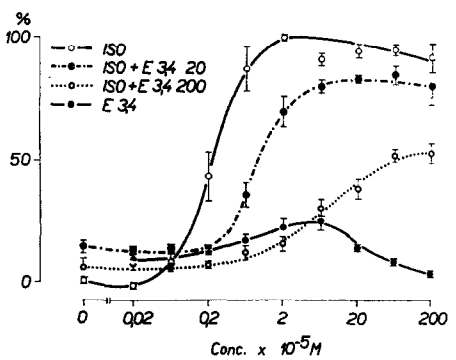

FIG. 2. Effects of E 3, 4 on the FFA mobilizing action of isoproterenol (ISO). Abscissa, molar concentration of the drug used as agonist; ordinate, intensity of FFA mobilization. 0 = basal release of FFA; 100% = release of FFA due to ISO (2×10^{-5} M). The circles and bars represent mean values ± S.E. Figures beside antagonist indicate concentration $\times 10^{-5}$ M

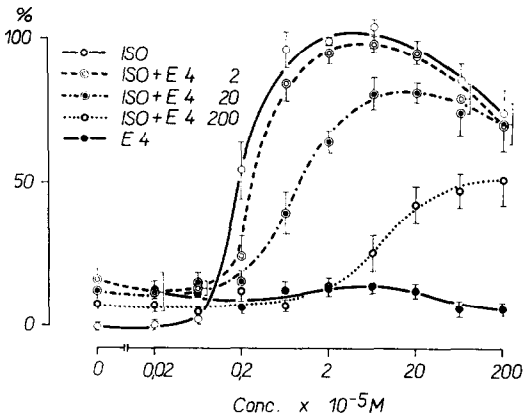

FIG. 3. Effects of E 4 on FFA mobilizing action of ISO. For legend see Fig. 2.

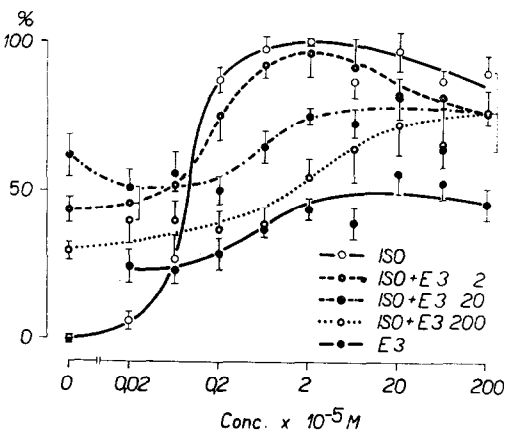

FIG. 4. Effects of E 3 on FFA mobilizing action of ISO. For legend see Fig. 2.

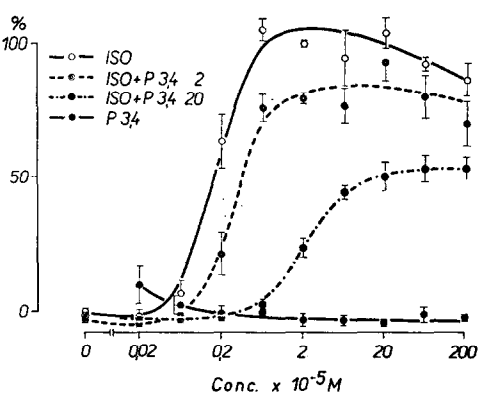

FIG. 5. Effects of P 3, 4 on FFA mobilizing action of ISO. For legend see Fig. 2.

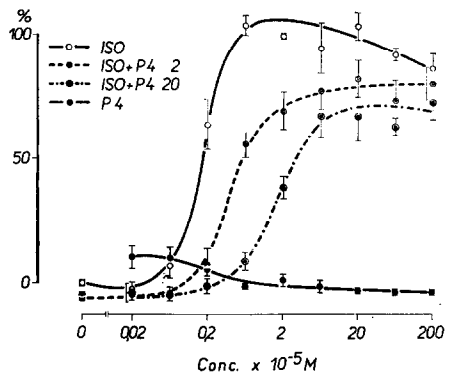

FIG. 6. Effects of P 4 on FFA mobilizing action of ISO. For legend see Fig. 2.

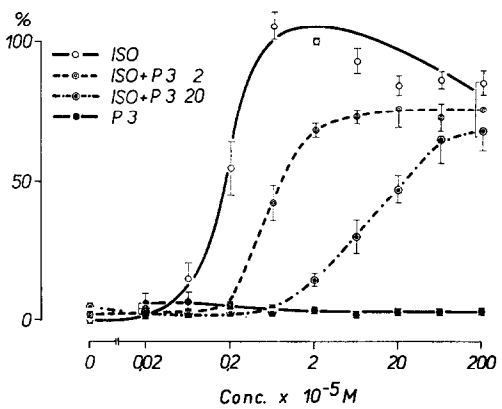

FIG. 7. Effects of P 3 on FFA mobilizing action of ISO. For legend see Fig. 2.

especially noted that no agonistic effect has been observed with P 3, that the agonistic effect is statistically insignificant in P 4 and P 3,4, and that, furthermore, the slight trace of mimetic action in the latter two drugs occurs at concentrations which are below those required in experiments demonstrating antagonistic effects. The displacement of the dose–response curves by compounds of the P series also indicates a combination of competitive and noncompetitive antagonism for these compounds.

Table 1 presents numerical values for the intrinsic activities and affinities calculated from the data obtained with each of the drugs. Also from this table, it is clearly evident that only in the E series has the agonistic action been found to be statistically significant. By lengthening the side-chain from an ethanol to a propanediol unit, the mimetic action disappears. Furthermore, the propanediol derivatives show an approximately 10-fold higher nonspecific antagonistic action than the corresponding ethanol derivatives.

Table 2 summarizes the parameters of specific competitive antagonism. It is evident that the substance P 3 is the most powerful antagonist of the two series of compounds tested. Also, when the pA_2 values of the corresponding E and P derivatives are compared, the P derivatives in each instance show a higher potency. In our experimental

TABLE 1. INTRINSIC ACTIVITY AND AFFINITY VALUES OF ADRENERGIC BLOCKING DRUGS STUDIED

Drug	Parameters of specific effects		Parameters of nonspecific effects	
	α	pD_2	pD'_2	pD''_2
E 3, 4	0·25 ($P < 0·02$)	5·3	2·5	2·6
E 4	0·15 ($P < 0·05$)		2·7	2·7
E 3	0·56 ($P < 0·001$)		2·2	2·2
P 3, 4	0·09 (NS)		3·7	3·7
P 4	0·11 (NS)		3·2	3·4
P 3	0·08 (NS)		3·3	3·5

TABLE 2. PARAMETERS OF SPECIFIC COMPETITIVE ANTAGONISM OF THE ADRENERGIC BLOCKING DRUGS STUDIED

Drug	pA_2 values calculated from effects of antagonist concentration		
	(2×10^{-5} M)	(2×10^{-4} M)	(2×10^{-3} M)
E 3, 4		4·2	4·3
E 4	4·6	4·2	4·3
E 3	4·6	4·5	4·1
P 3, 4	4·5	4·8	
P 4	4·9	4·6	
P 3	5·0	5·4	

series, therefore, the replacement of the ethanol chain by a propanediol chain leads to: (1) the abolition of undesired mimetic effect of the antagonist, (2) an increased potency as a specific antagonist, (3) an increased potency as a nonspecific antagonist.

When the pA_2 values are calculated by using data from experiments with different concentrations of the same blocking agent, then in the cases of E 3 and E 4, and less clearly also in the case of P 4, a progressive decrease of the affinity parameter occurs parallel with the increase in the concentration used. In spite of this, it remains clearly obvious that at the same concentration the pA_2 value of the corresponding P derivative is higher than that of the corresponding E derivative.

DISCUSSION

The intrinsic agonistic effects of some β-blockers with an ethanol side-chain are well known. For example, with regard to DCI, this has been shown for FFA mobilization Mühlbachová *et al.*,[4] Ashmore *et al.*,[5] Engelhardt,[6] Mühlbachová *et al.*,[7] Rudman *et al.*,[8] and many others and for the effects on the myocardium by Fleming and Hawkins.[9] Agonistic actions of FFA mobilization are also inherent in dichloronoradrenaline,[1] dichloroadrenaline,[1,10] pronethalol and some dihalogenated ethanol derivatives.[10] They have not been described as yet for MJ-1998 and MJ-1999, but to our knowledge no sufficiently detailed experiments have been carried out with these drugs. With the possible exception of these two substances therefore, the ethanol derivatives appear as "dualists", or more descriptively as "dualists with multiple actions", since the non-competitive component of antagonism has been found in every case in which the experimental detail was sufficient to explore this question.[7,11,12]

In marked contrast to the ethanol derivatives, all propanediol derivatives studied to date have failed to show any mimetic action of lipid mobilization. Moreover, in

unpublished experiments from our laboratory, no FFA mobilizing effect was found in propranolol.

The published reports together with the results reported in the present paper seem to indicate that insertion of the oxymethylene group between the phenyl ring and the ethanolamine side-chain diminishes or abolishes the intrinsic activity of a compound. Obviously, it is advantageous that blocking agents possess no intrinsic agonistic effect as well as a higher degree of noncompetitive antagonistic effect. The drugs of the P series studied under our experimental conditions, therefore, appear as "pure" (competitive and noncompetitive) antagonists without mimetic actions.

Of most importance, naturally, is the degree of competitive antagonism. In this regard, the P series is superior to the E series of drugs, as can be shown by a comparison of the effects of identical concentrations of the antagonists. On the other hand, our results leave some doubt as to whether it is appropriate to calculate in the usual manner the pA_2 value for drugs affecting adrenergic FFA mobilization. The gradual changes of the pA_2 values in 6 of 9 drugs—the 3 chlorophenyl compounds reported in the previous paper[1] and the 3 methylphenyl compounds reported here—seem to indicate that the standard formula applied to calculate the pA_2 value is not able in these cases to give reliable information about this parameter, which must be related to the stable dissociation constant of the drug–receptor complex. The valid quantitative laws for this situation may be different from that presumed by this formula. In further work it is suggested that the explanation may involve the existence of a "quadratic" dose–response relation, which relates to a "two-receptor" or "two-step" reaction type as described for some β-sympathomimetics affecting FFA mobilization.[13]

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