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ANTAGONISTIC EFFECTS OF THREE DICHLOROPHENYLETHANOLAMINES (DICHLORONORADRENALINE, DICHLOROADRENALINE AND DICHLOROISOPROTERENOL) ON ISOPROTERENOL-INDUCED FREE FATTY ACID MOBILIZATION

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Abstract—In experiments on rat epididymal adipose tissue *in vitro*, the influence of the adrenergic blocking agents, dichloronoradrenaline (DCN), dichloroadrenaline (DCA) and dichloroisoproterenol (DCI), on free fatty acid mobilizing effects of isoproterenol was determined quantitatively and the dose–response curves were analyzed.

All three drugs were found to act as "dualists with multiple action," i.e. weak agonists with combined competitive and noncompetitive antagonistic effects.

The competitive antagonistic activity increases in the sequence DCN < DCA < DCI, thus showing a structure–activity relationship typical of a β receptor. The affinities for the mimetic component of action also show the same order.

The noncompetitive, nonspecific component of the antagonistic actions shows no clear relation to the N-substituent.

Although the competitive antagonistic action is graduated in the sequence DCN < DCA < DCI, unexpectedly, the pA_2 value calculated for each concentration of the antagonist did not remain constant.

ALTHOUGH catecholamine-induced free fatty acid (FFA) mobilization has, in general, the characteristics of a function mediated by β-adrenergic receptors,[1] only very small differences in affinities among sympathomimetics of the catecholamine series have been found.[2] In a series of sympathomimetics in which the N-substituent is varied from — H to iPr, the range of affinities was only about twofold in experiments on FFA mobilization in rats *in vivo*[3] or in rat adipose tissue *in vitro*.[3, 4] In contrast, in the relaxation of tracheal muscle, selected as a typical function mediated by β-adrenergic receptors, the potencies of these sympathomimetics show about 300-fold differences.[5] Among the sympathomimetic catecholamines, the influence of changes in the N-substitution seems to be directed by different laws in respect to FFA mobilization than in respect to a typical smooth muscle β-adrenergic receptor.

In the present study, the influence of changes in the N-substituent was determined for β-adrenergic blocking agents in which the basic structure is 3′,4′-dichlorophenylethanolamine.

MATERIALS AND METHODS

White male rats of the Konarovice strain, weighing 180–220 g, were used. They were maintained on a standard diet until food was removed 24 hr before the start of the experiment. They were killed by decapitation. Epididymal fat pads of several animals were removed, cut in small pieces and mixed thoroughly. From this pooled material, samples of about 200 mg each were transferred to a 20-fold amount of incubation medium which contained 5% human albumin in Krebs–Ringer phosphate buffer (pH 7·4) and the appropriate drugs in various concentrations and combinations. The tissue suspensions were incubated at 37° for 90 min with gentle shaking.

The FFA mobilizing effect was estimated by using Dole's[6] method to determine the FFA released into the medium. The effects of all drug concentrations and combinations presented in a single figure were determined simultaneously, each experiment being repeated eight times.

Dose–response curves were evaluated according to the method of van Rossum,[7] in order to obtain values for the specific component of action (α, agonist activity; pD_2, a logarithmic function indicating affinity of the agonist; pA_2, a logarithmic function indicating affinity of the competitive antogonist) as well as for the nonspecific component of antagonistic action [pD'_2, noncompetive antagonism, and pD''_2, which is pI_2 only for the nonspecific component, i.e. the negative logarithm of the concentration at which the drug diminishes the agonist action to 50 per cent purely by its nonspecific, noncompetitive effect (for details see Wenke[8])].

The following drugs were used: DL-isoproterenol sulfate (ISO); DL-3′,4′-dichloronoradrenaline hydrochloride (DCN); DL-3′,4′-dichloroadrenaline hydrochloride (DCA); DL-3′,4′-dichloroisoproterenol sulfate (DCI).

RESULTS

The individual effects of DCN, DCA and DCI on FFA mobilization are compared with the effect of ISO in Fig. 1. Each of the three dichloroethanolamines showed an

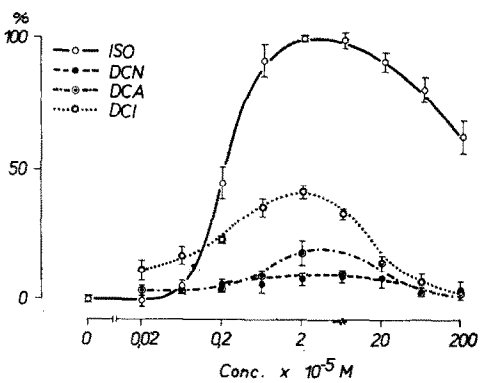

FIG. 1. Agonistic (FFA mobilizing) actions of DCN, DCA and DCI in comparison with the effects of ISO. Abscissa, molar concentration of the drug used as agonist; ordinate, intensity of FFA mobilization. 0 = basal release of FFA; 100% = release of FFA due to ISO in the concentration 2×10^{-5} M. Mean values ± S.E. For abbreviations, see text.

intrinsic agonistic effect with the following order of relative potencies: DCI>DCA> DCN. The influence of various concentrations of DCN, DCA and DCI on the dose-response curve of ISO is shown in Figs. 2–4. In each of these compounds a competitive component of antagonism against ISO is present as shown by the parallel shift of the curves to the right, but there is also a nonspecific antagonism as indicated by the depression of the maxima of the curves.

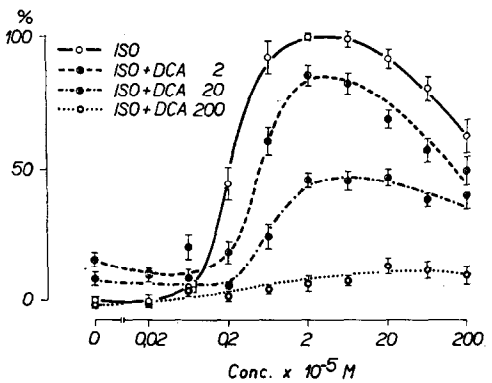

FIG. 2. Antagonistic effects of DCA on FFA mobilizing actions of ISO. Abscissa, molar concentration of ISO; ordinate, see legend to Fig. 1. Concentrations of antagonist (2, 20 and 200) $\times 10^{-5}$ M.

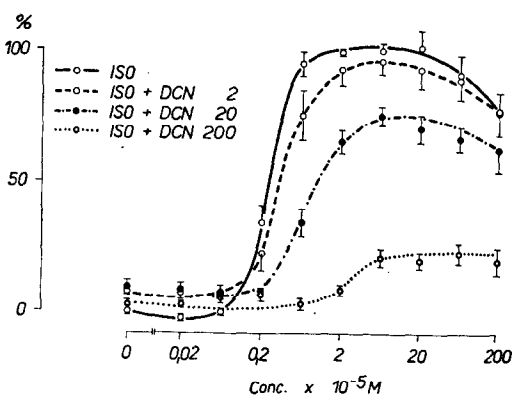

FIG. 3. Antagonistic effects of DCN on FFA mobilizing actions of ISO. For legend see Fig. 2.

Numerical data for the activities and affinities of the antagonists are given in Tables 1 and 2. A marked increase in intrinsic activity, i.e. the FFA mobilizing effect, occurs as the N-substituent changes in the sequence H < Me < iPr. The corresponding affinity parameters of this agonistic component of action could be estimated only in DCA and DCI; here also, the pD_2 values increase in the direction Me < iPr. In the specific actions given in Table 1, therefore, there is a trend clearly characteristic of the β-adrenergic receptor. The nonspecific, noncompetitive component of the antagonistic action, on the other hand, does not show any clear change related to the N-substituent.

Table 2 contains the values of the specific competitive antagonistic actions of the three β-adrenergic blocking agents. The pA_2 values are determined from the degree

of parallel shift evoked by each concentration of the antagonist. Here, in general, an increase of the affinities in the order H < Me < iPr corresponds to the order expected for affinity to β-receptors; but it must be noted that the resulting pA_2 values gradually

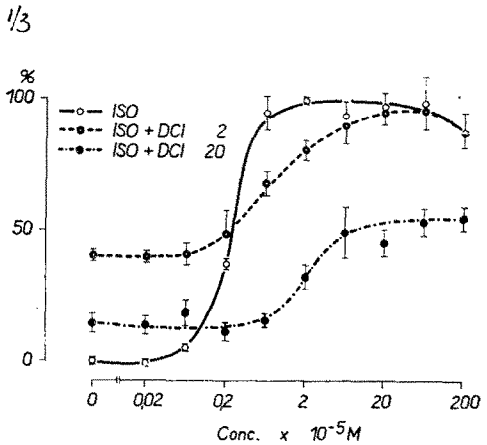

FIG. 4. Antagonistic effects of DCI on FFA mobilizing actions of ISO. For legend see Fig. 2.

TABLE 1. INTRINSIC ACTIVITIES AND AFFINITIES OF THE DICHLORINATED COMPOUNDS AS LIPID MOBILIZERS*

	Parameters of specific effects		Parameters of nonspecific effects	
Compound	α	pD_2	pD'_2	pD''_2
DCN	0·09		3·2	3·2
DCA	0·18	4·7	3·8	3·8
DCI	0·43	5·9	3·3	3·6

* For abbreviations and definition of parameters, see 'Materials and Methods .

TABLE 2. CALCULATED AFFINITIES OF THE DICHLORINATED COMPOUNDS AS ANTAGONISTS*

	pA_2 calculated from effects of antagonist concentration		
Compound	(2×10^{-5} M)	(2×10^{-4} M)	(2×10^{-3} M)
DCN	4·4	4·1	3·7
DCA	4·8	4·1	3·9
DCI	4·9	4·6	

* For abbreviations and definition of pA_2, see 'Materials and Methods'.

decrease with increasing concentration of each antagonist, i.e. the pA_2 values calculated for DCA at the concentrations 2×10^{-5} M and 2×10^{-3} M differ almost 10-fold. In spite of this complication, the sequence of each of the specific parameters determined is in agreement with an action of adrenergic receptors of the β-type.

DISCUSSION

The combination of actions of the investigated dichlorophenylethanolamines as agonists and as antagonists with competitive as well as noncompetitive characteristics makes them appear as "dualists with multiple actions", according to van Rossum's classification.[7] This is in full agreement with observations reviewed elsewhere.[8] The specific (agonistic and antagonistic) component of action shows a clear relation to the length of the N-substituent (H < Me < iPr) and thus corresponds to the structure–activity relationship well known for β-adrenotropic drugs. Quantitatively, however, a very peculiar situation occurs when the pA_2 values are calculated from effects of different concentrations of the same drug. The pA_2 value, which represents the negative logarithm of the dissociation constant of the drug–receptor complex, should be constant, of course, without regard to the concentration of the antagonist. Since in the reported experiments an increase in the concentration of the antagonist did not evoke a corresponding increase in antagonism, it seems obvious that the theoretical assumption concerning the relationship between the drug concentration and the final receptor attachment does not agree with the observed results. Thus, not only in catecholamine agonists, but also in dichlorinated antagonists, quantitative peculiarities were found which do not correspond to the dose–response relation of a simple β-adrenergic reaction.

A more precise analysis of the structure–function relationship has been made with β-adrenergic blocking agents with different substituents on the 3′ and 4′ positions of the ring. These studies will be presented in a subsequent paper.

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