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The guinea-pig trachea *O*-methylating system is more effective in modulating β_2- than β_1-adrenoceptor-mediated responses to isoprenaline

J. PROENÇA*, M. Q. PAIVA, S. GUIMARÃES, **Department of Pharmacology, Faculty of Pharmacy and Department of Pharmacology, Faculty of Medicine, 4200-Porto, Portugal*

Abstract—Assuming that responses of the guinea-pig trachea to isoprenaline in the presence of atenolol (10 μmol L^{-1}) are exclusively, or at least predominantly, β_2-adrenoceptor mediated and that responses to isoprenaline in the presence of ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxyl)-3-isopropylaminobutan-2-ol) (1 nmol L^{-1}) are exclusively, or at least predominantly β_1-adrenoceptor mediated, the influence of inhibition of COMT by U-0521 (dehydroxy-2-methyl propiophenone) (50 μmol L^{-1}) has been compared in both conditions. U-0521 enhanced β_2-adrenoceptor mediated responses to isoprenaline 3·3-fold, while those mediated by β_1-adrenoceptors were enhanced only 2·2-fold. It is concluded that in guinea-pig trachea COMT activity is functionally more effective in modulating responses which are mediated by β_2-adrenoceptors than responses mediated by β_1-adrenoceptors.

In vascular tissue and for a given dose, the concentration of noradrenaline and adrenaline available for their α-adrenoceptor effects is mainly governed by uptake into the sympathetic nerve terminals, while *O*-methylation is the main factor determining the concentration of those agonists available for the β-effect (Guimarães 1982).

On the other hand, it has been suggested that the β_1-adrenoceptor subtype responds primarily to neurotransmitter and is therefore innervated, whereas the β_2-adrenoceptor is non-innervated and responsive to circulating catecholamines (Ariëns & Simonis 1976; Russel & Moran 1980; Bryan et al 1981; Broadley et al 1984).

The present investigation was undertaken to compare the influence of the *O*-methylating system on responses mediated by β_1- and β_2-adrenoceptors in the guinea-pig trachea—a tissue where both β_1- and β_2-adrenoceptors exist (Furchgott 1976; O'Donnell & Wanstall 1979).

Correspondence to: S. Guimarães, Dept of Pharmacology, Faculty of Medicine, 4200 Porto, Portugal.

Materials and methods

Guinea-pigs, 320–460 g, were killed by a blow on the head and bled. The trachea was removed and placed in bubbled Krebs-Henseleit solution, cleaned of excess tissue and cut spirally (Constantine 1965). Each strip was approximately halved and each half suspended in a 25 mL organ bath containing Krebs solution with added EDTA (27 μmol L^{-1}) and ascorbic acid (56 μmol L^{-1}) saturated with 95% O_2 + 5% CO_2. Preparations were contracted with 0·3 μmol L^{-1} carbachol and relaxation responses were recorded by means of an isotonic myograph transducer, model MK II ser. 175, and amplifier, model CA 200, on a Physiograph DMP 4A (Narco Byosystems). The tension used was of about 1 g.

Cumulative concentration-response curves to isoprenaline were determined by the method of stepwise cumulative addition of the agonist. The concentration of the agonist in the bathing solution was increased 3-fold at each step, with each addition being made only after the response to the previous addition had

attained a maximal level and remained steady. After completion of a concentration-response curve, drugs were washed out from the preparation. The influence of U-0521 (dihydroxy-2-methyl propiophenone) (50 μmol L^{-1}) on responses to isoprenaline was determined in the presence of either atenolol (10 μmol L^{-1}) or ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxyl)-3-isopropylaminobutan-2-ol) (1 nmol L^{-1}). Atenolol and ICI 118,551 were added to the bath 30 min before the addition of the agonist. U-0521 was added to the bath 20 min before the agonist (10 min after the β-blocker).

Values presented in the text are geometric means with 95% confidence limits. The significance of differences between means was calculated by Student's *t*-test. *P* values of 0·05 or less were considered significant.

Results and discussion

The maximal response to isoprenaline (relaxation) inhibited 85 ± 7% of the previous contraction caused by carbachol. Either atenolol or ICI 118,551 shifted the concentration-response curve of isoprenaline to the right in a parallel way. Table 1 summarizes the results.

Table 1. Influence of U-0521 (inhibition of COMT) on responses to isoprenaline obtained either in the presence of atenolol (A) or ICI 118,551 (B). The enhancement of the response to isoprenaline caused by U-0521 was more marked in the presence of atenolol than in the presence of ICI 118,551.

	A		B
	EC50 of isoprenaline (nmol L^{-1})		EC50 of isoprenaline (nmol L^{-1})
Control	5·8 (2·2–15·3)	Control	4·2 (1·9–9·2)
In the presence of atenolol (10 μmol L^{-1})	29·8[a] (10·0–88·6)	In the presence of ICI 118,551 (1 nmol L^{-1})	23·3[b] (9·8–55·8)
In the presence of atenolol (10 μmol L^{-1}) + U-0521 (50 μmol L^{-1})	9·0[a'] (3·9–20·7)	In the presence of ICI 118,551 (1 nmol L^{-1}) + U-0521 (50 μmol L^{-1})	10·4[b'] (4·7–23·0)
Ratio a/a'	3·3 (2·9–3·7)	Ratio b/b'	2·2 (1·7–2·8)
n =	6		6

The difference between the ratios a/a' and b/b' was significant ($P < 0·05$).

U-0521, an inhibitor of COMT (Giles & Miller 1967; Guimarães et al 1978), caused parallel displacements of the concentration-response curves of isoprenaline to the left, either in the presence of atenolol or ICI 118,551. The displacements to the left caused by U-0521 in the presence of atenolol were significantly larger than those obtained in the presence of ICI 118,551.

Assuming that responses to isoprenaline in the presence of atenolol (10 μmol L^{-1}) are exclusively, or at least predominantly β_2-adrenoceptor-mediated and that responses to isoprenaline in the presence of ICI 118,551 (1 nmol L^{-1}) are exclusively, or at least predominantly, β_1-adrenoceptor mediated (O'Donnell & Wanstall 1981) it can be concluded that U-0521 enhanced β_2-adrenoceptor-mediated responses more than responses mediated by β_1-adrenoceptors. How can this differential influence of U-0521 on the effects of isoprenaline be explained? The deviation supersensitivity caused by inhibition of a saturable site of loss depends on the maximum possible degree of supersensitivity, on the ratio (K_m of the site of loss)/(EC50 of the agonist), and on the distance between the site of loss and the receptors (Trendelenburg 1972; Guimarães & Paiva 1984; Paiva & Guimarães 1984; Guimarães & Trendelenburg 1985). Since the EC50 of isoprenaline for β_1- and β_2-adrenoceptors is almost the same and the K_m of COMT is the same, only a different distance between the site of loss and the two different subtypes of β-adrenoceptors involved in the responses can account for the different degree of supersensitivity observed.

In fact, the concentrations of atenolol and ICI 118,551 used in this study caused equal degrees of antagonism of isoprenaline.

In both conditions the EC50 of isoprenaline was largely below the K_m of COMT thus allowing maximal supersensitivity to develop as a consequence of inhibition of COMT. Hence, there was no danger that COMT activity was closer to saturation in one series of experiments than in the other series. Accordingly we concluded that the distance between COMT sites and β_1-adrenoceptors is larger than that between COMT sites and β_2-adrenoceptors. Hence, COMT activity is functionally more effective in modulating responses which are mediated by β_2-adrenoceptors than responses mediated by β_1-adrenoceptors.

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