Differential influence of block of catechol-*O*-methyl transferase (COMT) activity and of neuronal uptake on α- and β-adrenergic effects

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Previously it has been shown in cat nictitating membrane and dog saphenous vein strips that block of catechol-*O*-methyl transferase(COMT) causes an increase in the tissue sensitivity to isoprenaline as well as an increase in the maximum relaxant effect produced by this amine (Trendelenburg, 1974; Guimarães, Azevedo & others, 1975). In both reports it was shown that, under control conditions, the concentration of isoprenaline required for maximum activation of β-adrenoceptors also activates α-adrenoceptors; thus the full relaxant effect of isoprenaline is partially masked by activation of α-adrenoceptors (contraction). It therefore seems reasonable to postulate that block of COMT enhances β- more than α-effects (Trendelenburg, 1974; Guimarães & others, 1975). But how is this achieved? Two hypotheses have been proposed: according to one it is held that β-effects are selectively enhanced by block of COMT because block of a 'site of loss' causes supersensitivity to the agonist when its effects are elicited by concentrations not saturating the 'site of loss'; this is so when isoprenaline acts on β-adrenoceptors (low concentrations) and not when it acts on the α-adrenoceptors (high concentrations). As a second hypothesis it was suggested that the concentration of COMT might be higher in the immediate neighbourhood of β-adrenoceptors than in the close vicinity of the α-adrenoceptors; in that case, block of COMT would enhance β- more than α-effects, thus allowing for the full relaxant effect of isoprenaline to develop before the opposite effect (due to α-stimulation) could appear.

Since in the tissues referred to above the ED50 of isoprenaline for β-effects is approximately 200 times lower than that for α-effects, we have repeated these experiments on a tissue possessing α- and β-adrenoceptors having similar sensitivity to the same agonist.

The rabbit intestine was chosen because the ED50 of noradrenaline for α-effects is very close to that for β-effects (Furchgott, 1960; Guimarães, 1968) and the ED50 for both effects is smaller than 10^{-6} M, a condition needed to obtain an increase in sensitivity to a catecholamine by block of a 'site of loss' (Trendelenburg, 1972).

Rabbit jejunal segments of about 3 cm length were used. Each was suspended in a 25 ml bath of Krebs-Henseleit solution at 37° aerated with 5% carbon dioxide in oxygen and connected to an isotonic lever with the weight adjusted so that tone was allowed to develop (Burn, 1952). Responses to sympathomimetic amines were measured in terms of the reduction in tone produced by the drugs, the magnitude of the response being the distance between midpoints of the rhythmic contractions before and after the drug (Lum, Kerman & Heilman, 1966).

To inhibit α- or β-adrenoceptors, piperoxan ($1{\cdot}7$–$6{\cdot}9 \times 10^{-5}$ M) and propranolol ($1{\cdot}8 \times 10^{-5}$ M), respectively, were used. Phenylephrine and isoprenaline were used as agonists for assessing blockade of α- and β-adrenoceptors.

Concentration-response curves, obtained by intermittent additions of the agonist were determined before and after treatment with U-0521 (dihydroxy-2-methyl propiophenone; 10^{-4} M) or cocaine ($1{\cdot}4 \times 10^{-5}$ M), and the effect caused by these drugs was taken as due to block of COMT or neuronal uptake, respectively.

Table 1 summarizes the results. Block of COMT with U-0521 enhanced to a greater extent the effects mediated through the β-adrenoceptors than those mediated through the α-adrenoceptors (Table 1, col. 4) while block of neuronal uptake by cocaine enhanced the effects mediated through the α-adrenoceptors more than those due to β-adrenoceptors (Table 1, col. 5).

Table 1. *ED50 values for (−)-noradrenaline, (−)-adrenaline, and (−)-isoprenaline after block of α-adrenoceptors and for noradrenaline and adrenaline, after block of β-adrenoceptors.* Also shown the influence of U-0521 (block of COMT) or cocaine (block of neuronal uptake) on β- or α-effects of those amines. The slopes of the concentration-response curves after the shift to the left (where it occurred) both after U-0521 and cocaine, were not significantly different from the control.

	Amine	ED50 (± s.e.) (control)	Ratio (to control ED50) of ED50 in the presence of:	
			U-0521	Cocaine
For β-effects (in the presence of piperoxan)	Noradrenaline	$2{\cdot}7 \pm 0{\cdot}2 \times 10^{-7}$ (n = 15)	$6{\cdot}0 \pm 0{\cdot}6^{I}$	$1{\cdot}3 \pm 0{\cdot}1^{V}$
	Adrenaline	$9{\cdot}0 \pm 0{\cdot}7 \times 10^{-7}$ (n = 15)	$5{\cdot}6 \pm 0{\cdot}7^{II}$	$1{\cdot}0 \pm 0{\cdot}1$
	Isoprenaline	$0{\cdot}9 \pm 0{\cdot}1 \times 10^{-7}$ (n = 8)	$10{\cdot}0 \pm 1{\cdot}5$	—
For α-effects (in the presence of propranolol)	Noradrenaline	$3{\cdot}4 \pm 0{\cdot}2 \times 10^{-7}$ (n = 13)	$1{\cdot}8 \pm 0{\cdot}2^{III}$	$2{\cdot}5 \pm 0{\cdot}3^{VI}$
	Adrenaline	$1{\cdot}80 \pm 0{\cdot}1 \times 10^{-7}$ (n = 13)	$1{\cdot}8 \pm 0{\cdot}2^{IV}$	$1{\cdot}2 \pm 0{\cdot}2$

Significance of difference between I and III ($P < 0{\cdot}001$); II and IV ($P < 0{\cdot}01$); V and VI ($P < 0{\cdot}05$).

* Correspondence.

How can this differential influence of U-0521 on the effects of noradrenaline and adrenaline and of cocaine on the effects of noradrenaline be explained? Three factors are known to account for the degree of supersensitivity to an agonist caused by block of a 'site of loss': (a) the affinity of the agonist for the receptors (Langer & Trendelenburg, 1969; Trendelenburg, 1972); (b) the affinity of the agonist for the 'site of loss' (Langer & Trendelenburg, 1969; Trendelenburg, 1972); (c) the distance between the 'site of loss' and the receptors (Bevan & Verity, 1967). The first factor (a) cannot be considered to explain our results because the affinity of noradrenaline for α- (pD_2 = 6·47; ED50 = $3·4 \times 10^{-7}$ M and for β-adrenoceptors (pD_2 = 6·57; ED50 = $2·7 \times 10^{-7}$ M) is almost the same in this preparation (see Table 1) and does not account for the large difference in the degree of sensitization, especially since the β-effect of adrenaline was much more enhanced by U-0521 than its α-effect (Table 1, col. 4) and since its affinity for α-adrenoceptors was 5 times higher than for β-adrenoceptors (Table 1, col. 3). Even if the 'real' affinity (after blockade of all 'sites of loss'; Guimarães, 1975) is determined, the values for α- and β-adrenoceptors are similar. In fact, calculation of the 'real' affinity (by dividing the values found by the enhancement caused by U-0521 + cocaine) results in the following values: 7·46 (ED50 = $3·5 \times 10^{-8}$ M) and 7·21 (ED50 = $7·5 \times 10^{-8}$M for β- and for α-adrenoceptors, respectively, in the case of noradrenaline and 6·80 (ED50 = $1·6 \times 10^{-7}$ M and 7·00 (ED50 = $1·0 \times 10^{-7}$ M), respectively, for adrenaline. The second factor (b), i.e., the affinity of the agonist for the 'site of loss' is not involved since this parameter has a constant value and the same agonist was used to activate both α- and β-adrenoceptors. Only the third factor (c) can explain the differential influence caused by block of a 'site of loss'.

Thus it appears likely that block of COMT by U-0521 enhances the β-effects of both noradrenaline and adrenaline more than their α-effects because COMT is more concentrated in the vicinity of β- than around α-adrenoceptors; on the other hand, block of neuronal uptake by cocaine enhances the α-effects of noradrenaline more than its β-effects because the α-adrenoceptors are more concentrated around the nerve terminals than are the β-adrenoceptors.

In the intestine there is good agreement between anatomical data and our pharmacological evidence: it is believed that α-adrenoceptors are associated with the many adrenergic fibres supplying the ganglia of the myenteric plexus, whereas β-adrenoceptors are closely related with muscle cells which are supplied by few adrenergic fibres (see review of Furness & Costa, 1974).

Recently Belfrage & Rosell (1976) assumed that vascular β-adrenoceptors were not closely related to the nerve terminals. In the present investigation strong evidence is presented to support the view that in the rabbit intestine there is a preferential distribution of α-adrenoceptors in relation to nerve terminals and of β-adrenoceptors in relation to COMT-containing cells.

If we consider the hitherto published evidence Guimarães, 1975; Guimarães & others, 1975; Belfrage & Rosell, 1976) and the present data, we can conclude that not only are α-adrenoceptors situated in close relation with the nerve terminals but also that β-adrenoceptors are situated nearer to COMT loci. This differential distribution of the adrenoceptors appears to be a characteristic of the different tissues studied.

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