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Effect of steroids on β-adrenoceptor-mediated relaxation of pig bronchus

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1 Progesterone, testosterone (40 μM), cortisol and cortisol hemisuccinate (80 μM) caused 6–8 fold potentiations of (±)-isoprenaline (Iso)-induced relaxations of pig bronchus while several other steroids caused smaller potentiations or had no effect.

2 17β-Oestradiol (40 μM) increased the potency of Iso, (−)-adrenaline (Adr) and (−)-noradrenaline (NA) by 10.6, 2.3 and 2.6 fold respectively but had no significant effect on the potency of fenoterol (Fen).

3 Inhibition of catechol-*O*-methyl transferase (COMT) with U-0521 (30 μM) caused a 6 fold increase in the potency of Iso but failed to alter the potency of Adr, NA or Fen. The extraneuronal uptake inhibitor normetanephrine (50 μM) caused significant 2 fold increases in the potency of Iso and Adr but did not potentiate the responses to NA or Fen.

4 In preparations where the potency of Iso had already been increased by U-0521 (30 μM) or by normetanephrine, 17β-oestradiol produced no significant further increase in potency. These results indicate that steroid-induced increases in the potency of catecholamines in pig bronchus can be explained in terms of inhibition of COMT or extraneuronal uptake or both.

Introduction

Various steroids potentiate the actions of catecholamines both *in vivo* (Fritz & Levine, 1951; Schmid, Eckstein & Abboud, 1967; Pun, McCulloch & Rand, 1973; Shenfield, Hodson, Clarke & Paterson, 1975; Goldie, 1980) and *in vitro* (Kalsner, 1969; Kaumann, 1972; Goldie, 1976; Cornish, Goldie & Miller, 1978; Hackney & Szentivanyi, 1980). The underlying mechanism of steroid-induced potentiation of catecholamine responses has been attributed variously to adrenoceptor sensitization (Besse & Bass, 1966; Yard & Kadowitz, 1972; Brine, Cornish & Miller, 1979), inhibition of catecholamine uptake (Kaumann, 1972; Pun *et al.*, 1973; Goldie, 1976) and inhibition of catechol-*O*-methyl transferase (COMT) (Kalsner, 1969; Cornish & Goldie, 1980). Indeed, each of these mechanisms may be involved depending upon the tissue, species and steroid used. Preparations of pig bronchus are being evaluated currently in our laboratory, as a model of human central airways. We have studied the effects of several steroids on catecholamine-induced relaxation of pig bronchus, with a view to clarifying the possible mechanism of steroid-induced potentiation of catecholamines reported in asthmatics (Shenfield *et al.*, 1975).

Methods

Bronchi (2–3 mm i.d.) were dissected from the central lobes of lungs taken from pigs freshly slaughtered at a local abattoir. Some bronchi were cut into helical strips suspended under 500 mg tension in Krebs Henseleit solution aerated with 5% CO_2 in O_2 at 37°C. Changes in isometric tension were monitored with a Grass force-displacement transducer (FTO3C) coupled to a preamplifier and a Rikadenki pen recorder (Model 1328L). After equilibrating for 60 min, preparations were contracted with carbachol (0.22 μM) as previously described (Goldie, Paterson & Wale, 1982) and cumulative concentration-effect curves to relaxant sympathomimetic amines were superimposed. Relaxations were measured as a % of the maximal response (E_{max} = 100%). The EC_{50} value obtained from the second control concentration-effect curve was taken as a measure of control bronchial sensitivity to a catecholamine. Additional concentration-effect curves were produced following equilibration of preparations for 60 min with one concentration of a steroid or normetanephrine (50 μM) to inhibit extraneuronal catecholamine uptake, or U-0521 (30 μM) to inhibit COMT. The ratio,

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Table 1 Effect of steroids on the sensitivity of isolated preparations of pig bronchus to isoprenaline (Iso)

Steroid (μM)	*Mean potentiation* ± s.e.mean	n
17β-Oestradiol-HS (40)	14.9 ± 1.4***	5
17β-Oestradiol (40)	10.6 ± 2.1***	11
Oestrone (40)	4.2 ± 1.1*	6
Oestriol (40)	2.6 ± 0.4†	4
Stilboestrol (40)	5.8 ± 1.5**	7
Progesterone (40)	7.9 ± 2.1***	4
Testosterone (40)	6.7 ± 1.2***	5
Cortisol-HS (80)	7.2 ± 1.8***	6
Cortisol (80)	7.7 ± 1.1***	6
Prednisolone-HS (80)	1.7 ± 0.2**	8
Prednisone-HS (80)	3.2 ± 0.4**	6
Dexamethasone-HS (80)	1.1 ± 0.3†	6
Cortisone-HS (80)	1.5 ± 0.2†	4
Corticosterone (40)	4.9 ± 1.1**	4
Deoxycorticosterone acetate (40)	3.5 ± 0.5**	7

Steroids were used as the base unless the hemisuccinate salt (HS) or other derivative is specified.
Potentiation = Iso EC_{50} control: Iso EC_{50} steroid.
Significant potentiation *$P<0.05$; **$P<0.01$; ***$P<0.001$. No significant difference †$P>0.05$; (paired t test).

EC_{50} control: EC_{50} test was taken as a measure of the drug-induced change in bronchial sensitivity to the sympathomimetic.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (±)-isoprenaline hydrochloride; carbamylcholine chloride (Sigma); fenoterol hydrobromide (Boehringer Ingelheim); ethanol (re-distilled; Colonial Sugar Refineries); normetanephrine hydrochloride (Calbiochem); base and sodium hemisuccinate (HS) derivatives of cortisol and 17β-oestradiol, corticosterone, cortisone-HS, deoxycorticosterone acetate, dexamethasone-HS, diethyl stilboestrol, oestriol, oestrone, prednisolone-HS, prednisone-HS, progesterone, testosterone (Steraloids Inc.); U-0521 (3,4,dihydroxy-2-methyl propriophenone (Upjohn). Doses refer to bath concentrations.

Results

Effects of steroids on isoprenaline-induced bronchial relaxation

None of the steroids had any significant effect upon the size of carbachol-induced (0.22 μM) contractions. However, in a few cases, carbachol contractions were poorly sustained in preparations exposed to 17β-oestradiol (40 μM). In such cases, initial levels of tone were re-established by carbachol titration and the stability of the contraction verified before relaxation responses to sympathomimetic amines were superimposed.

17β-Oestradiol and 17β-oestradiol hemisuccinate (HS) (40 μM) caused significant mean (± s.e.mean) leftward shifts in Iso concentration-effect curves of 10.6 ± 2.1 ($n=11$) and 14.9 ± 1.4 ($n=5$) fold re-

Table 2 Effect of U-0521, normetanephrine or 17β-oestradiol on the sensitivity of pig isolated bronchus to sympathomimetic amines

	Iso	*Adr*	*NA*	*Fen*
EC_{50} *control* (μM)	0.2 ± 0.01 (109)	4.2 ± 0.5 (28)	1.7 ± 0.2 (26)	6.1 ± 0.7 (40)
		Ratio $\frac{EC_{50}\ control}{EC_{50}\ test}$		
Test drug				
U-0521 30 μM	6.0 ± 0.8*** (14)	2.0 ± 0.5† (6)	1.5 ± 0.4† (8)	1.3 ± 0.2† (4)
U-0521 60 μM	4.2 ± 0.8** (5)			
Normetanephrine 50 μM	2.0 ± 0.2** (13)	1.9 ± 0.4* (8)	2.5 ± 0.9† (7)	0.6 ± 0.1* (4)
17β-Oestradiol 40 μM	10.6 ± 2.1*** (11)	2.3 ± 0.2** (9)	2.6 ± 0.3*** (8)	2.4 ± 0.5† (6)

Data are presented as mean ± s.e.mean.
Numbers in parentheses indicate the number of experiments.
Significant increase or decrease in bronchial sensitivity to a relaxant amine is indicated viz: $*P<0.05$; $**P<0.01$; $***P<0.001$. No significant increase $\dagger P>0.05$ (Paired *t* test).

spectively (Table 1). These potentiations did not differ significantly from one another ($P>0.2$, non-paired *t* test). In addition, approximately 6–8 fold increases in Iso potency were observed following pretreatment with progesterone, testosterone, stilboestrol (40 μM), cortisol and cortisol-HS (80 μM), while smaller but still statistically significant potentiations were observed in the presence of oestrone, corticosterone, deoxycorticosterone (40 μM), prednisolone-HS and prednisone-HS (80 μM) (Table 1). Conversely, oestriol (40 μM), dexamethasone-HS and cortisone-HS (80 μM) failed to alter significantly the potency of Iso in pig bronchus (Table 1). Ethanol (17.4 mM), which was used to solubilize base derivatives of steroids, had no significant effect on responses to Iso ($P>0.1$; paired *t* test).

Effect of 17β-oestradiol, inhibition of uptake or O-methylation on responses to catecholamines and to fenoterol

The COMT inhibitor U-0521 (30 μM), potentiated responses to Iso by 6 fold but failed to alter the relaxant potency of Adr, NA or Fen. (Table 2). U-0521 (60 μM) also potentiated responses to Iso by 4.2 fold. The extraneuronal uptake inhibitor, normetanephrine (50 μM), caused 2 fold increases in the potency of Iso and Adr, failed to affect responses to NA significantly and significantly decreased the potency of Fen by 1.6 fold. 17β-Oestradiol (40 μM) which potentiated responses to Iso by 10.6 fold, also increased the potency of Adr and NA by 2.3 and 2.6 fold respectively, but did not significantly increase the potency of Fen (Table 2). With the exception of the base and hemisuccinate derivative of 17β-oestradiol, none of the steroids caused a significantly different potentiation of Iso-induced bronchial relaxation from that caused by U-0521 (30 μM) ($P>0.2$; non-paired *t* test).

In some experiments, the potency of Iso was determined in preparations pretreated with either normetanephrine (50 μM) or with U-0521 (30 μM) and again later in the combined presence of normetanephrine (50 μM) and 17β-oestradiol (40 μM) or U-0521 (30 μM) and 17β-oestradiol (40 μM). In these cases, 17β-oestradiol (40 μM) failed to increase further the potency of Iso ($P>0.05$). Furthermore, U-0521 (30 μM) caused no further increase in the potency of Iso in preparations pretreated with normetanephrine (50 μM).

Theophylline (0.01–2 mM) also caused concentration-related relaxations of pig bronchus. The mean EC_{50} value was 0.23 ± 0.03 mM ($n=6$). 17β-Oestradiol (40 μM) caused a small but significant increase in theophylline potency (1.6 ± 0.2 fold; $n=5$; $0.02<P<0.05$).

Discussion

High concentrations of 17β-oestradiol and some related oestrogenic steroids as well as progesterone, testosterone, corticosterone (40 μM), cortisol-HS (80 μM) caused more than 4 fold increases in the relaxant potency of Iso in pig bronchus (Table 1). The mineralocorticoid, deoxycorticosterone acetate (40 μM), and the glucocorticoids, prednisolone-HS and prednisone-HS (80 μM), also produced smaller

but significant potentiations. Thus, there is no correlation between the effects of these different steroids on Iso responses in pig isolated bronchus and their other well known effects *in vivo*. Potentiations caused by 17β-oestradiol and cortisol were not significantly different from those produced by their respective water soluble hemisuccinate derivatives. Thus, lipid solubility does not seem to be a major factor in determining the extent of steroid-induced potentiation of catecholamine responses.

17β-Oestradiol, progesterone, testosterone, corticosterone, deoxycorticosterone and cortisol-HS are potent inhibitors of extraneuronal catecholamine uptake (Kalsner, 1969; Salt, 1972; Cornish *et al.*, 1978) Of these steroids, only 17β-oestradiol is known also to inhibit the neuronal uptake of catecholamines (Iversen & Salt, 1970; Salt, 1972; Cornish *et al.*, 1978). Normetanephrine (4–22 μM) is known to inhibit selectively extraneuronal uptake by approximately 50% (Burgen & Iversen, 1965; Mireyless & Foster, 1973; Major, Sauerwein & Graefe, 1978). Table 2 shows that normetanephrine (50 μM) caused only a 2 fold potentiation of Iso responses. However, the potency of Fen was reduced by nearly 2 fold. This was probably due to the β-adrenoceptor blocking activity of normetanephrine (Goldie & Paterson, 1982). Thus, the extent of normetanephrine-induced potentiations of Iso, Adr and NA (Table 2) and therefore the significance of extraneuronal uptake, may be under-estimated. Only 17β-oestradiol and its hemisuccinate derivative caused significantly greater potentiations of Iso than did a maximally effective concentration of the COMT inhibitor U-0521 (30 μM). Thus, steroid-induced increases in the potency of Iso may be caused by direct inhibition of COMT and/or by reduced access of Iso to intracellular COMT. This suggestion is consistent with results showing that 17β-oestradiol had no significant effect on the potency of the non-catechol β-adrenoceptor agonist Fen and with the fact that 17β-oestradiol failed to alter further the potency of Iso in bronchial preparations pretreated with either U-0521 or normetanephrine. Furthermore, both U-0521 and 17β-oestradiol had much smaller effects on the potency of Adr and Nor than of Iso. This is to be expected given that Iso is the preferred substrate for both extraneuronal uptake and for COMT (Gillespie, 1973; Guldberg & Marsden, 1975; Trendelenburg, 1980). Kalsner (1969) suggested that cortisol-induced potentiation of vascular responses to catecholamines may be due to inhibition of COMT or extraneuronal uptake. This steroid was shown to inhibit both COMT and uptake in cat heart (Cornish & Goldie, 1980).

Alternatively, steroid-induced alteration of β-adrenoceptor function has been postulated to explain enhanced catecholamine potency (Besse & Bass, 1966; Yard & Kadowitz, 1972; Brine *et al.*, 1979). It has been shown that increasing the fluidity of cell membranes increases adenyl cyclase activity (Rimon, Hanski, Braun & Levitski, 1978; Bakardjieva, Galla, Helmreich & Levitski, 1979). Furthermore, steroids such as cortisol have been shown to enhance adenyl cyclase activity *per se* (Marone, Lichtenstein & Plaut, 1980) and to restore depressed adenyl cyclase activity in leukocytes from asthmatics (Logsdon, Middleton & Coffey, 1972). In addition, cortisol potentiated Iso-induced increases in adenyl cyclase activity in leukocytes from healthy volunteers (Marone *et al.*, 1980). It is possible therefore, that 17β-oestradiol and related steroids increased the fluidity of plasma membranes in central airways smooth muscle of the pig, causing potentiation of catecholamine-induced increases in adenyl cyclase activity which in turn resulted in potentiation of catecholamine-induced bronchial relaxation. However, as previously indicated, responses to Iso were potentiated by 17β-oestradiol to a significantly greater extent than those to either Adr or NA while responses to Fen were unaffected. Furthermore, the relaxant potency of the phosphodiesterase inhibitor theophylline was only enhanced 1.6 fold by 17β-oestradiol (40 μM). Such selectivity is not compatible with steroid-induced sensitization of β-adrenoceptors or increased adenyl cyclase activity.

Hemisuccinate or phosphate salts of glucocorticoids such as prednisolone and dexamethasone are useful in the treatment of asthma (Collins, Harris, Clark & Townsend, 1970; Ellul-Micallef & Fenech, 1975; Ellul-Micallef, Borthwick & McHardy, 1980). However, massive concentrations of these steroids failed to increase more than marginally the sensitivity of pig isolated bronchus to Iso. In addition, the control potency of Iso in pig bronchus was more than 10 times less than in human bronchi (Goldie *et al.*, 1982). Thus, isolated pig bronchus may not be an appropriate model of human central airways. However, steroid hormones clearly increase the potency of catecholamines in pig bronchus by inhibition of catecholamine metabolism or uptake or both.

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