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Effect of hypothermia on β_1-adrenoceptor-mediated relaxation of pig bronchus

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1 The relaxant potencies of (±)-isoprenaline and of (−)-noradrenaline (NA) in the pig isolated bronchus were increased 5.4 and 3.1 fold respectively by lowering the organ bath temperature from 37°C to 27°C, whereas the potencies of the non-catecholamine β-adrenoceptor agonists fenoterol and orciprenaline were not significantly changed.

2 At 37°C, the catechol-*O*-methyl transferase (COMT) inhibitor U-0521 (30 μM), caused a 7.2 fold increase in the potency of isoprenaline but had no effect on the potency of fenoterol. At 27°C the potency of isoprenaline was similar in the absence or presence of U-0521 (30 μM). Furthermore, in bronchi where extraneuronal uptake was inhibited by phenoxybenzamine, the potency of NA was not significantly altered by reducing bathing temperature from 37°C to 27°C.

3 These results suggest that the hypothermic potentiation of isoprenaline in pig bronchus resulted from inhibition of COMT or of access to COMT, rather than from sensitization of β_1-adrenoceptors.

Introduction

Williams & Broadley (1982) have shown that reducing bath temperature from 38°C to 30°C causes potentiation of β_1-adrenoceptor mediated responses of guinea-pig atria, papillary muscles and ileum, but not of responses of guinea-pig lung strip or uterus where β_2-adrenoceptors predominate (O'Donnell, Persson & Wanstall, 1978; Siegl, Rossi & Orzechowski, 1979). Therefore, it is possible that hypothermia selectively alters the affinity of agonists for β_1-adrenoceptors (Broadley & Williams, 1982). Inhibition of catecholamine uptake processes or of catechol-*O*-methyl transferase (COMT) is also known to cause potentiation of responses of isolated airways and cardiac muscle preparations to β-adrenoceptor agonists (Foster, 1967; 1968; 1969; Kaumann, 1972; Cornish, Goldie & Miller, 1978; Goldie, 1980; Wanstall & O'Donnell, 1981). Potentiation of responses of guinea-pig trachea and of mouse, rat, guinea-pig and cat atria caused by lowering the bath temperature by as much as 10°C from approximately 37°C (Schneider & Gillis, 1966; Foster, 1967; Munoz-Ramirez, Haavik & Ryan, 1973; Kunos & Nickerson, 1977; Oppermann, Ryan & Haavik, 1972) has also been attributed to inhibition of catecholamine uptake processes or of COMT (Foster, 1969; Sachs, 1970; Oppermann, Ryan & Haavik, 1971; 1972; Cornish *et al.*, 1978; Bönisch, Uhlig & Trendelenburg, 1979).

In the present study, we have examined the effects of reduced bath temperature on the potency of β-adrenoceptor agonists in pig isolated bronchus, a preparation which contains predominantly β_1-adrenoceptors (Goldie, Paterson & Wale, 1983). The non-selective agonists isoprenaline and orciprenaline were used as well as the relatively selective β_1-agonist noradrenaline (NA) and the β_2-selective amine fenoterol. It was anticipated that the use of β-agonists which are substrates for extraneuronal uptake and *O*-methylation (e.g. isoprenaline and NA) as well as amines with lesser affinity for uptake and which are not substrates for COMT (e.g. fenoterol and orciprenaline), would indicate the relative importance of inhibition of uptake processes and changes in agonist affinity in hypothermic potentiation of β_1-adrenoceptor-mediated responses.

Methods

Bronchi (2–3 mm i.d.) were dissected from macroscopically normal lobes of lung from pigs freshly slaughtered at a local abattoir. Bronchi were cut into spiral strips and suspended under 500 mg tension in

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Krebs-Henseleit solution maintained at 37°C and aerated with 5% CO_2 in oxygen. Preparations were contracted with carbachol (0.22 μM) and relaxation responses to cumulative additions of isoprenaline, NA, fenoterol or orciprenaline were superimposed as previously described (Goldie, Paterson & Wale, 1982). All responses were calculated as a % of the maximal amine-induced relaxation. The EC_{50} value from the second control curve was taken as a control measurement of amine potency. Preparations were then equilibrated for 1 h at 27°C before the potency of isoprenaline, NA, fenoterol or orciprenaline was again determined by similar cumulative addition of the drug. The effect of temperature reduction to 27°C on the potency of isoprenaline and fenoterol was also determined in pig bronchi pretreated for 1 h with the COMT inhibitor U-0521 (30 μM). Similarly, the potency of isoprenaline was determined at both temperatures in preparations pretreated for 1 h with cortisol (80 μM).

In a separate series of experiments, pig bronchial preparations were first exposed to phenoxybenzamine (Pbz) (50 μM) for 30 min followed by washout at 10 min intervals for the next 30 min. This procedure was carried out at 37°C and has been shown to cause complete, non-competitive inhibition of extraneuronal uptake (O'Donnell & Wanstall, 1976). Pbz is also known to inhibit neuronal uptake non-competitively at this concentration (Iversen & Langer, 1969; Sanchez-Garcia, Garcia, Martinez-Sierra & Valasco-Martin, 1977). Preparations were then exposed to K^+ (30 mM) which caused submaximal contraction, and cumulative concentration-effect curves to NA were then superimposed at 37°C and later at 27°C as described above.

The effect of temperature reduction to 27°C on cumulative concentration-effect curves to carbachol was also examined.

Drugs used were (±)-isoprenaline hydrochloride, carbamylcholine chloride (Sigma); fenoterol hydrobromide (Boehringer Ingelheim); orciprenaline hydrochloride (I.C.I.); U-0521 (3,4-dihydroxy-2-methyl propriophenone) (Upjohn). Solutions of isoprenaline, NA, fenoterol and orciprenaline were prepared in saline (0.9% w/v NaCl solution) containing ascorbic acid 20 μg ml^{-1}.

Results

The potencies of isoprenaline and of NA in pig bronchus were increased 5.4 fold and 3.1 fold respectively when the bath temperature was lowered from 37°C to 27°C, although the potencies of fenoterol and orciprenaline were not significantly altered by temperature reduction ($P > 0.2$; Student's paired t test) (Table 1). Each of these agonists caused complete relaxation of carbachol-induced tone at both 37°C and 27°C. When the organ bath temperature was reduced from 27°C to 22°C, the potency of isoprenaline was not significantly increased further

Table 1 Effects of temperature reduction on the relaxant potency (EC_{50}) of isoprenaline (Iso), fenoterol (Fen), orciprenaline (Orci) and noradrenaline (NA) in pig bronchus

	EC_{50} (M)			
	Iso	*Fen*	*Orci*	*NA*
Untreated				
37°C	$2.96 \pm 0.49 \times 10^{-7}$ (8)	$4.34 \pm 1.28 \times 10^{-6}$ (7)	$5.44 \pm 0.61 \times 10^{-6}$ (7)	$1.41 \pm 0.19 \times 10^{-6}$ (10)
27°C	$0.55 \pm 0.08 \times 10^{-7}$*** (8)	$3.30 \pm 1.06 \times 10^{-6}$ (7)	$5.53 \pm 1.15 \times 10^{-6}$ (7)	$0.46 \pm 0.09 \times 10^{-6}$*** (10)
22°C	$0.43 \pm 0.11 \times 10^{-7}$ (5)			
U-0521 (30 μM)				
37°C	$0.41 \pm 0.09 \times 10^{-7}$ (7)	$5.15 \pm 2.02 \times 10^{-6}$ (7)		
27°C	$0.31 \pm 0.06 \times 10^{-7}$ (7)	$5.77 \pm 2.06 \times 10^{-6}$ (7)		
Cortisol (80 μM)				
37°C	$0.34 \pm 0.05 \times 10^{-7}$ (7)			
27°C	$0.24 \pm 0.04 \times 10^{-7}$ (7)			

Results are presented as mean ± s.e.mean.
Numbers in parentheses indicate the number of experiments.
Significantly lower EC_{50} value at 27°C cf. 37°C is indicated viz. ***$P < 0.001$ (Student's paired t test).

($P > 0.2$, Student's paired t test, $n = 5$). Pretreatment with U-0521 (30 μM) at 37°C caused a 7.2 fold increase in the potency of isoprenaline but did not significantly alter the potency of fenoterol. Lowering the temperature to 27°C in U-0521-treated strips, failed to alter further the potency of either isoprenaline or fenoterol. Cortisol (80 μM) significantly increased the potency of isoprenaline at 37°C by 8.7 fold. Subsequent lowering of the bath temperature to 27°C caused no further significant change in potency.

In Pbz pre-treated bronchi, NA caused a mean (± s.e.mean) reduction in K^+-induced tone at 37°C of 74 ± 5.6%. This value was reduced to 48.6 ± 6.1% at 27°C. The mean EC_{50} values at these temperatures were $4.70 \pm 0.61 \times 10^{-7}$ M and $4.57 \pm 0.75 \times 10^{-7}$ M respectively. These values are not significantly different ($P > 0.2$, Student's paired t test, $n = 6$).

At 37°C the mean EC_{50} value for the bronchial spasmogen carbachol was 1.99 ± 0.29 μM ($n = 11$). At 27°C the EC_{50} value for carbachol was 1.45 ± 0.25 μM ($n = 6$). These values were not significantly different ($P > 0.2$, Student's non-paired t test). Furthermore, the response to carbachol (0.22 μM) as a % of the maximal response, was not significantly different at these two temperatures ($P > 0.2$).

Discussion

If hypothermia-induced potentiation of β-adrenoceptor-mediated responses of isolated tissue preparations was mainly caused by a selective increase in the affinity of β-agonists for β_1-adrenoceptors (Broadley & Williams, 1982), then the potency of all β-agonists would be expected to be increased. In the present study in pig bronchus, which contains a predominance of β_1-adrenoceptors (Goldie *et al.*, 1983), this was not observed. Reducing the organ bath temperature from 37°C to 27°C increased the potency of the non-selective catecholamine isoprenaline by 5.4 fold and caused a smaller (3.1 fold) increase in the potency of the relatively β_1-selective catecholamine NA. Conversely, the potencies of the non-catecholamines fenoterol (β_2-selective) and orciprenaline were unaltered.

We have previously shown that the COMT inhibitor U-0521 (30 μM) increased the potency of isoprenaline by about 6 fold but failed to alter the potencies of fenoterol or NA (Foster *et al.*, 1983). It was also shown that cortisol (80 μM) caused a 7 fold increase in isoprenaline potency in pig bronchus and that this was best explained in terms of inhibition of COMT and/or of extraneuronal uptake. In the present study, reducing the organ bath temperature from 37°C to 27°C did not cause further increases in the potency of isoprenaline in bronchi pretreated with either U-0521 (30 μM) or cortisol (80 μM). This is consistent with the known inhibitory effects of the latter on extraneuronal uptake and on COMT (Cornish *et al.*, 1978; Cornish & Goldie, 1980). Similarly, in K^+-depolarized pig bronchus, where extraneuronal uptake was inhibited with Pbz, hypothermia failed significantly to alter the potency of NA.

Williams & Broadley (1982) showed that orciprenaline-induced responses of isolated guinea-pig atria, papillary muscles and ileum (β_1-adrenoceptors), pretreated with metanephrine (10 μM) to inhibit extraneuronal uptake, were potentiated 3–9 fold when the temperature of the bathing solution was reduced from 38°C to 30°C, while responses of guinea-pig uterus and lung strip (β_2-adrenoceptors) were not affected. It was suggested that the reduction in temperature caused a selective alteration in β_1-adrenoceptor function (Broadley & Williams, 1982). While changes in β-adrenoceptor function do not adequately explain the present results, it is equally difficult to explain the results obtained by Broadley & Williams (1982) in terms of inhibition of amine uptake or of COMT. Extraneuronal uptake is a relatively weak process in guinea-pig heart (Uhlig, Bönisch & Trendelenburg, 1974). Even isoprenaline, which has a high affinity for this pathway in most tissues is not avidly transported in guinea-pig heart (Trendelenburg, 1980). Thus, it would be expected that the non-catecholamine β-agonist orciprenaline would be even less avidly sequestered extraneuronally. In addition, Broadley & Williams (1982) pretreated guinea-pig atria and ileal preparations with metanephrine to ensure that extraneuronal uptake was inhibited before the bathing temperature was reduced. Perhaps orciprenaline was transported into a compartment in these tissues which was not inhibited by metanephrine.

The results of the present study suggest that hypothermia-induced potentiation of responses of isolated tissue preparations to catecholamines is mainly caused by inhibition of intracellular amine uptake or metabolism (Schneider & Gillis, 1966; Oppermann *et al.*, 1972; Munoz-Ramirez *et al.*, 1973). Thus, the potencies of fenoterol and of orciprenaline were not altered by temperature reduction from 37°C to 27°C because these agonists are not *O*-methylated by COMT. We have previously shown that the potency of NA was not significantly increased after inhibition of COMT (Foster *et al.*, 1983) or following inhibition of neuronal uptake with cocaine (10 μM) (Goldie *et al.*, 1983). In this study the small but statistically significant increase in NA potency may have resulted from simultaneous hypothermia-induced inhibition of both neuronal and extraneuronal uptake. In line with this is the fact

that the potency of NA was not altered by temperature reduction in bronchi where uptake processes were inhibited by Pbz pretreatment. Certainly the present data are not compatible with the concept of sensitization of β_1-adrenoceptors (Broadley & Williams, 1982). Caron & Lefkowitz (1974) also concluded that the characteristics of cardiac β-adrenoceptors were not altered over a wide range of temperatures.

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