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Hexoprenaline: β-adrenoreceptor selectivity in isolated tissues from the guinea-pig

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SUMMARY

1. A catecholamine β-adrenoreceptor agonist, hexoprenaline, was examined *in vitro* on five guinea-pig tissues and its potency relative to isoprenaline (as 100) obtained.

2. Hexoprenaline clearly delineated between those tissues classified as containing β_2-adrenoreceptors (trachea, hind limb blood vessels and uterus; relative potencies 219, 110 and 76 respectively) and those classified as containing β_1-adrenoreceptors (atria and ileum; relative potencies 3·3 and 1·0 respectively).

3. Hexoprenaline differed from some previously studied noncatecholamine β-adrenoreceptor agonists in being only two-fold less potent, relative to isoprenaline, as a vasodilator in perfused hind limb than as a tracheal relaxant.

Key words: β-adrenoreceptors, blood vessels, bronchodilators, guinea-pig, hexoprenaline, selectivity, trachea.

INTRODUCTION

In a previous study using *in vitro* preparations from the guinea-pig, O'Donnell & Wanstall (1974a) examined potential sympathomimetic bronchodilator compounds for their potency as tracheal relaxants (β_2-adrenoreceptors), atrial stimulants (β_1-adrenoreceptors) and vasodilators in the perfused hind limb (β_2-adrenoreceptors). Some resorcinolamines showed selectivity for trachea compared with not only atria but also blood vessels. There is some evidence in dogs *in vivo* that other noncatecholamines, namely carbuterol (Wardell *et al.*, 1974), and salbutamol and terbutaline (Wasserman & Levy, 1974), display similar selectivity. If selectivity between respiratory and vascular smooth muscle represents selectivity at the β-adrenoreceptor level, it poses the question whether the β_1/β_2 subclassification (Lands *et al.*, 1967a; Lands, Luduena & Buzzo, 1967b) is too rigid. Recently Spilker, McKeon & Arnold (1974) suggested that the use of noncatecholamine sympathomimetics to delineate

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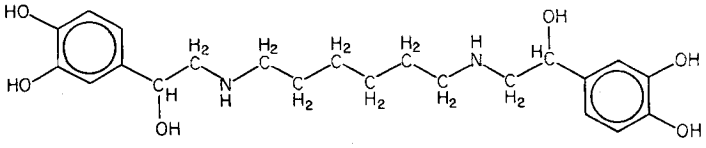

FIG. 1. Structure of hexoprenaline.

β_1- and β_2-adrenoreceptors in tissues is not valid since, by definition, α- β_1- and β_2-adrenoreceptors are based on the responsiveness of the receptor sites to certain catecholamines. We were therefore interested to examine how closely clinically used selective bronchodilators which *are* catecholamines fit the β_1/β_2 subclassification. This paper describes our findings with a recently introduced bronchodilator, hexoprenaline (Fig. 1), which has a catechol nucleus and has been described as selective in its action on β_2-adrenoreceptors (Stormann, 1970; Turnheim & Kraupp, 1971; Stormann & Turnheim, 1973). A preliminary report of this work has been presented to the Australian Physiological and Pharmacological Society (O'Donnell & Wanstall, 1974b).

METHODS

Female guinea-pigs weighing 340–750 g were used.

Tracheal chains, atria and perfused hind limbs

Four-ring tracheal chain preparations, spontaneously beating atria and single isolated perfused hind limbs were set up as described previously (O'Donnell & Wanstall, 1974a). Concentrations or doses of drugs producing 50% of the maximum response to isoprenaline (EC_{50}, ED_{50}) were obtained as described by O'Donnell & Wanstall (1974a).

Uterus

Drug induced relaxation of the isolated uterus from guinea-pigs in dioestrus was determined using a method based on that of Wasserman & Levy (1972) for rat uterus. A small length of uterus was set up in de Jalon's solution at 27°C aerated with 5% CO_2 in oxygen. Under these conditions the uteri were quiescent. Isotonic contractions to acetylcholine were recorded using a modified strain gauge. Sub-maximal contractions to acetylcholine (0·02 to 0·2 μg/ml in different experiments) were obtained at 4 min intervals. A time of 1–1·5 min was required for each response to acetylcholine to reach maximum. Concentrations of relaxant drug were in contact with the preparation for 2·5 min before adding acetylcholine. This contact time was sufficient for maximal inhibitory responses to be reached. The response was measured as percentage inhibition of the standard acetylcholine contraction. At least three concentrations of test drug and isoprenaline were examined on each preparation. The concentration producing 50% inhibition (EC_{50}) was interpolated from each concentration-response line.

Electrically stimulated ileum

Segments of non-terminal ileum, taken from guinea-pigs pretreated with reserpine (5 mg/kg i.p., 24 h previously) to avoid release of noradrenaline following electrical stimulation, were set up in Krebs solution containing ascorbic acid (200 μg/ml) maintained at

30–32°C and aerated with 5% CO_2 in oxygen. The ileum was stimulated by means of two platinum field electrodes. Single pulses of supramaximal voltage and 2 ms duration were applied at a rate of 0·1 Hz; isotonic contractions were recorded using a modified strain gauge. Constant contractions could be obtained for several hours and these were inhibited on addition of isoprenaline or hexoprenaline. Each concentration of drug was left in contact with the tissue until the inhibitory response was complete. The response to electrical stimulation was rapidly restored after the drug was washed out of the bath. Thus it was not necessary to use cumulative addition of the drugs.

In this preparation, concentration-response curves to isoprenaline comprised three distinct phases, each of which occurred over a particular concentration range. Between 1 nM and 100 nM graded inhibition of the contractions occurred. Between 100 nM and 2 μM the inhibition remained at a plateau (from 29 to 40% inhibition in different experiments). Above 2 μM further graded inhibition occurred, reaching 100% at 100 μM. Inhibitions produced by low concentrations of isoprenaline (1 nM to 100 nM) were unaffected by phentolamine (100 nM) but the concentration range over which the inhibition remained at a plateau was extended to 50 μM. Thus the concentrations required to produce complete inhibition were ten- to 100-fold higher in the presence than in the absence of phentolamine. This suggests that α-adrenoreceptors are involved in the inhibitory response produced with high concentrations of isoprenaline. Thus, to obtain the β-adrenoreceptor mediated response to isoprenaline without interference due to α-adrenoreceptor stimulation, phentolamine (100 nM) was included in the Krebs solution and the degree of inhibition at which the plateau occurred was taken as the maximum (100%) response. Responses to isoprenaline and hexoprenaline were expressed as a percentage of this maximum response and the EC_{50} interpolated from each concentration-response line. The isoprenaline concentration range for β-adrenoreceptor stimulation and the extent of the inhibition agree closely with those already described by Kosterlitz, Lydon & Watt (1970).

Experimental design

The order of obtaining concentration-response lines to isoprenaline and hexoprenaline was randomized between experiments. In some experiments a second concentration-response line to hexoprenaline was obtained in the presence of propranolol (100 nM for 60 min) or cocaine (10 μM for 30 min). Additional experiments were carried out on preparations of trachea, atria, hind-limb and uterus taken from guinea-pigs pretreated with reserpine (5 mg/kg i.p., 24 h previously) and of ileum taken from non-reserpinized guinea-pigs.

Mean negative log EC_{50} values (trachea, atria, uterus and ileum), and mean negative log ED_{50} values (hind limb) were calculated from at least five experiments for each tissue. These values were used as a measure of the absolute potency of the drugs. In addition, the relative potency of hexoprenaline compared with isoprenaline (arbitrarily assigned a value of 100) on each tissue was calculated as follows: log potency ratios from each individual experiment were calculated by subtracting the negative log EC_{50} for isoprenaline from that for hexoprenaline. The relative potency of hexoprenaline (isoprenaline = 100) was calculated as (100 × antilog mean log potency ratio).

Drugs

Acetylcholine (Sigma); cocaine hydrochloride (Drug Houses of Australia); hexoprenaline sulphate (Chemie-Linz); (±)-isoprenaline sulphate (Burroughs Wellcome); phentolamine

methanesulphonate (Regitine, Ciba); (±)-propranolol hydrochloride (ICI); reserpine (Serpasil, Ciba). All drugs were obtained as pure powders except for phentolamine and reserpine which were obtained as solutions in ampoules.

Statistical analyses

The measure of variation of the mean quoted is the standard error (s.e.m.). The significance of the difference in potency between hexoprenaline and isoprenaline was assessed using a *t*-test on paired neg log EC_{50} values from individual experiments (paired *t*-test).

RESULTS

On all the tissues hexoprenaline, like isoprenaline, produced the expected responses to β-adrenoreceptor stimulation, i.e. relaxation of trachea, increase in atrial rate, vasodilatation in the perfused hind limb, inhibition of acetylcholine-induced contractions of the uterus and inhibition of electrically induced contractions of the ileum. Hexoprenaline produced the same maximum response as isoprenaline on each tissue and its concentration-response lines were parallel to those of isoprenaline. On trachea, atria, uterus and ileum propranolol (100 nM) caused a parallel shift of the concentration-response lines to a higher concentration range and on the hind limb a dose of propranolol (10 nmol) abolished the vasodilator response to hexoprenaline. Responses to hexoprenaline were not potentiated by cocaine

TABLE 1. Mean potencies expressed as the mean negative log of the concentrations or doses of hexoprenaline (hexo) or isoprenaline (iso) producing 50% of the maximal responses (i.e. EC_{50} or ED_{50}), in guinea-pig isolated trachea, hind limb, uterus, atria and ileum. Mean log potency ratios are mean (neg log EC_{50} or ED_{50} (hexo) minus neg log EC_{50} or ED_{50} (iso)). Relative potency of hexoprenaline (iso = 100) equals (100 × antilog mean log potency ratio). All mean values are shown with s.e.m. in parentheses. For potency values, *n* = number of preparations; for mean log potency ratios, *n* = number of paired observations.

	Mean Potency		Mean log potency ratio	Relative potency (iso = 100)
	Hexoprenaline	Isoprenaline		
Trachea	8·40 (0·13)	8·07 (0·04)	0·34 (0·10)*	219
(EC_{50})	*n* = 8	*n* = 8	*n* = 8	
Hind Limb	10·06 (0·14)	10·02 (0·18)	0·04 (0·05)	110
(ED_{50})	*n* = 5	*n* = 5	*n* = 5	
Uterus	8·01 (0·08)	8·12 (0·09)	−0·12 (0·05)	76
(EC_{50})	*n* = 5	*n* = 5	*n* = 5	
Atria	7·14 (0·06)	8·62 (0·04)	−1·48 (0·05)†	3·3
(EC_{50})	*n* = 7	*n* = 7	*n* = 7	
Ileum	6·07 (0·07)	8·08 (0·07)	−2·00 (0·08)†	1
(EC_{50})	*n* = 5	*n* = 5	*n* = 5	

* Hexoprenaline significantly more potent than isoprenaline $0·05 > P > 0·01$ (paired *t*-test).

† Hexoprenaline significantly less potent than isoprenaline $P < 0·001$ (paired *t*-test).

(10 μM). Hexoprenaline was four to five times more potent on preparations of uterus and hind limb taken from reserpinized guinea-pigs than on those from untreated guinea-pigs but a similar increase in potency was also seen with isoprenaline. On trachea, atria and ileum no difference between preparations from reserpinized and untreated guinea-pigs was observed. The above observations provide evidence that the responses produced by hexoprenaline in the five tissues studied are predominantly due to a direct action on β-adrenoreceptors and that it does not appear to have an affinity for neuronal uptake. The possibility of an affinity for extraneuronal uptake has not been excluded.

Hexoprenaline had a slower onset of action than isoprenaline on trachea, atria and ileum, but this was not apparent on uterus or hind limb. Hexoprenaline was readily removed from the tissues on washing, but the resting state of tracheal and atrial preparations took slightly longer to restore than after isoprenaline.

Table 1 shows the negative log EC_{50} (or ED_{50}) values of hexoprenaline and isoprenaline and the relative potency of hexoprenaline with respect to isoprenaline (= 100) on each of the five tissues. Hexoprenaline was twice as potent as isoprenaline on trachea ($0{\cdot}05>P>0{\cdot}01$; paired t-test; 7 d.f.), equipotent with isoprenaline on hind limb and uterus ($P>0{\cdot}05$; paired t-test; 4 d.f.) and significantly less potent than isoprenaline on atria ($P<0{\cdot}001$; paired t-test; 6 d.f.) and ileum ($P<0{\cdot}001$; paired t-test; 4 d.f.). Thus the relative potencies on trachea (219), hind limb (110) and uterus (76) are markedly higher than those on atria (3·3) and ileum (1·0). There was only a two-fold difference between the relative potency of hexoprenaline on trachea and hind limb.

DISCUSSION

In the present study hexoprenaline was examined *in vitro* on preparations of five different tissues from guinea-pigs. There was a marked difference between the relative potencies of hexoprenaline on respiratory smooth muscle (trachea), uterus and hind limb blood vessels and the relative potencies on atria and ileum. In other words hexoprenaline displayed a selectivity for those tissues which were classified by Lands *et al.* (1967a, b) as containing β_2-adrenoreceptors.

The majority of sympathomimetic amines which have previously been shown to be selective for β_2-adrenoreceptors have been derived by modification of the noradrenaline molecule in one or more of the following ways: (a) the introduction of a large, branched substituent on the amine group, (b) a change in the ring structure so that the compound is no longer a catecholamine, (c) substitution of an alkyl group on the α-carbon atom. The most selective sympathomimetic bronchodilators in clinical use possess both a branched substituent on the amine group and also either a different ring structure to noradrenaline, for example salbutamol, terbutaline and fenoterol, or a substituent group on the α-carbon, for example isoetharine. Hexoprenaline retains the catechol ring structure and has no α-carbon substituent yet it displays a selectivity, *in vitro* at least, comparable with that of fenoterol, salbutamol and isoetharine. In other *in vitro* studies (Lands *et al.*, 1967a; O'Donnell & Wanstall, 1974a) the introduction of only a large branched substituent on the amine group of the noradrenaline molecule has not conferred selectivity of the magnitude observed for hexoprenaline in this study. Therefore, it seems unlikely that it is simply the bulk of the N-substituent group which is important for the selectivity of the hexoprenaline molecule for β_2-adrenoreceptors.

Hexoprenaline has been described as having a long duration of action *in vivo*. This has been attributed partly to the fact that one product of O-methylation, the mono-O-methoxy metabolite, has itself considerable sympathomimetic activity, and partly to strong tissue binding of the molecule (Stormann & Turnheim, 1973). In the present *in vitro* experiments, hexoprenaline was readily removed from the preparations which suggests that it is not strongly bound. Its long duration of action *in vivo* therefore probably reflects the sympathomimetic activity of the mono-O-methoxy metabolite.

Although Stormann & Turnheim (1973) described hexoprenaline as a selective β_2-adrenoreceptor agonist, they noted that some of their results were not in close agreement with the β_1/β_2 scheme. For example, there was a greater than ten-fold difference between the relative potencies of hexoprenaline, with respect to isoprenaline, on two tissues containing β_2-adrenoreceptors, i.e. uterus (rat) and trachea (calf). Similarly, there was at least a ten-fold difference between the relative potencies of hexoprenaline on two tissues containing β_1-adrenoreceptors, i.e. intestine (rabbit) and heart (guinea-pig). The discrepancies may result from their use of tissues from four different species, since in the present investigation in which only guinea-pig tissues were used, differences of the magnitude noted by Stormann & Turnheim (1973) were not seen between tissues within either the β_2 group (trachea, uterus and hind limb blood vessels) or the β_1 group (atria and ileum).

Hexoprenaline was only two-fold less potent as a vasodilator than as a tracheal relaxant. In this respect it differed from some β_2-adrenoreceptor-selective resorcinolamines, including the clinically used bronchodilators fenoterol (Th1165a) and terbutaline (Me501). Fenoterol was eleven-fold and terbutaline eighteen-fold less potent (relative to isoprenaline) as a vasodilator than as a tracheal relaxant (O'Donnell & Wanstall, 1974a). The clinical importance of vascular side effects in sympathomimetic bronchodilators is not clearly defined. However, if the *in vitro* model is a guide to possible *in vivo* effects, then our findings suggest that, in the doses required to produce bronchodilatation, hexoprenaline might be expected to cause more pronounced peripheral vasodilatation than either fenoterol or terbutaline.

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