4 h and was not used within 30 min of collection. Aggregation was followed with a Born Mk III aggregometer and the viability of the platelets was tested by constructing a dose-response curve to ADP-induced aggregation at the beginning and the end of each experiment. Acetone was used as the vehicle for the daphnane esters and at a maximum concentration of 0·5% v/v in PRP it did not induce aggregation or modify the ADP-induced aggregation. The esters were tested in a concentration range of 0·01 to 70 μM. The 50% irritant dose (ID50) of the esters in the mouse ear erythema test was also determined as previously described (Evans & Schmidt 1979).

Resiniferatoxin and tinyatoxin in doses of up to 70 μM did not cause aggregation of human blood platelets (Fig. 1). ROP, which differs from the other compounds in that it has a free C-20 hydroxy group, caused a slow irreversible aggregation of human platelets in doses of from 7 to 70 μM. A maximum aggregation of only 27% was produced which could not be inhibited by indomethacin, indicating that the characteristic platelet release reaction did not occur. In the erythema test, resiniferatoxin and tinyatoxin were potent inflammatory agents with ID50's of 0·00021 and 0·0012 nM respectively. ROP was about 7×10^3 times less potent than resiniferatoxin and 1×10^3 times less potent than tinyatoxin (Fig. 1) in this test.

These findings add further support to our previous conclusions (Westwick et al 1979) that a C-20 primary hydroxy group on the nucleus is necessary for aggregating activity on human platelets, whereas erythema of the mouse ear is only quantitatively affected with the structural change in the molecule.

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β-Adrenoceptor mediated actions of RO363 and (−)-isoprenaline in anaesthetized cats, rats and rabbits

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RO363 [(±)-1-(3,4-dimethoxyphenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol)] is a recently developed directly acting β-adrenoceptor stimulant, which, on the basis of in vitro studies shows a high degree of selectivity for β_1-adrenoceptors (Iakovidis et al 1979). Preliminary studies in anaesthetized cats (Raper et al 1978) showed that RO363 was approximately half as potent as (−)-isoprenaline in eliciting increases in heart rate and more than 100 times less potent as a vasodilator in the hindlimb vasculature. In the present experiments the in vivo activity of RO363 has been further investigated in anaesthetized cats, rats and rabbits.

Arterial blood pressure and heart rate were recorded in artificially respired, bilaterally vagotomized cats (bethanidine not given) and rats anaesthetized with chloralose (80 mg kg^{-1} + pentobarbitone sodium 6 mg kg^{-1}) and urethane (1·25 g kg^{-1}) respectively, and in spontaneously breathing sodium pentobarbitone (35 mg kg^{-1}) anaesthetized rabbits. Constant dose-response curves to intravenous (−)-isoprenaline were first obtained and thereafter responses to RO363 were monitored.

In all three species RO363 and (−)-isoprenaline produced positive chronotropic effects and vasodepressor responses that were antagonized by propranolol (0·5 mg kg^{-1}). Dose-response curves to the two catecholamines were parallel and similar maximal responses were obtained. The duration of the responses to RO363 were 2–3 times longer than those to (−)-isoprenaline (Fig. 1). In cats, RO363 produced small, propranolol sensitive, increases in blood pressure at low doses, while higher doses produced depressor responses

Table 1. In vivo comparisons of the activities of RO363 and (−)-isoprenaline. Mean i.v. ED50 doses ($\times 10^{-10}$ mol kg^{-1}) ± s.e.m. are shown for the production of (−)-isoprenaline (ISO) and RO363-induced increases in heart rate (HR), decreases in diastolic blood pressure (DBP) and soleus muscle contractility (Soleus), and inhibition of 5-HT-induced bronchoconstriction (BC). Doses quoted for decreases in hindlimb perfusion pressure (HLPP) are those required to produce a reduction of 30 mmHg following intra-arterial administration of the compounds. Mean relative potencies from individual experiments (± s.e.m.) are also shown (ED50 RO363: ED50 (−)-isoprenaline).

	n	ED50 (ISO)	ED50 (RO363)	Relative Potency
Cat, HR	6	2·3±0·7	5·9±0·8	2·8±0·7
Rat, HR	4	0·7±0·3	1·0±0·4	1·7±0·4
Rabbit, HR	4	3·4±1·0	24±3	8·5±1·4
Cat, DBP	7	3·3±1·7	75±31	28±6
Rat, DBP	3	5·6±3·2	141±8	24±2
Rabbit, DBP	4	3·0±1·2	96±39	34±10
Cat, BC	7	0·8±0·1	30±5	42±10
Cat, Soleus	6	1·7±0·2	157±30	95±17
Cat, HLPP	8	0·12±0·03	35±22	225±88

* Correspondence.

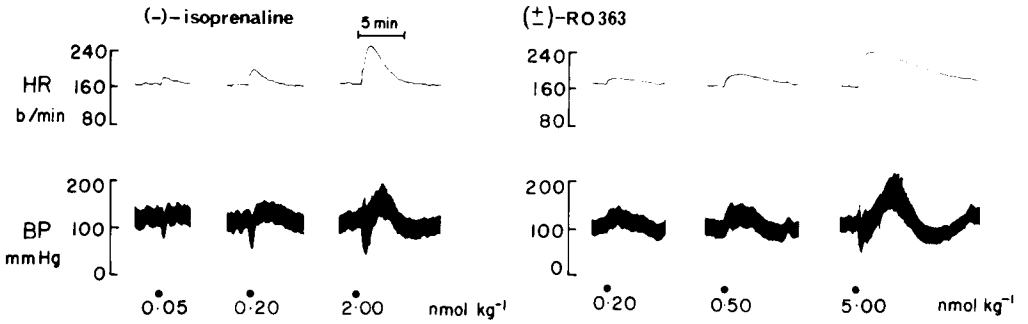

FIG. 1. Effects of i.v. (−)-isoprenaline and (±)-RO363 on the heart rate (HR, beats per min) and arterial blood pressure (BP) of anaesthetized cats.

(Fig. 1). This pressor activity was not seen in rabbits or rats. RO363 was 2–8 times less potent than (−)-isoprenaline as a cardiac stimulant and 25–30 times less potent in reducing diastolic blood pressure (Table 1). In cats and rats, the threshold doses of RO363 required to produce vasodepressor activity elicited maximal chronotropic effects, while in rabbits this differential activity was less marked. This is presumably due to the lower cardiac potency of RO363 in this species.

The above results suggest that RO363 displays β_1-adrenoceptor selectivity in vivo, however, the selectivity shown is less than that shown in vitro, where the compound is approximately half as potent as (−)-isoprenaline at β_1- and 100–300 times less active at β_2-adrenoceptors. This may be a reflection of the many factors that can potentially affect diastolic blood pressure, a feature which reduces the suitability of this parameter as a measure for β_2-receptor mediated activity.

Further experiments were therefore performed to assess the actions of RO363 in systems where effects are thought to be due to β_2-receptor stimulation. Decreases in soleus muscle contractility and hindlimb perfusion pressure, and inhibition of 5-HT-induced increases in intra-tracheal pressure were assessed in anaesthetized cats as described by Dowd et al (1977). In all three tissues, dose-response lines to RO363 and (−)-isoprenaline were parallel and similar maximal responses were obtained. In the soleus muscle and in the hindlimb vasculature, RO363 was 100–200 times less potent than (−)-isoprenaline, whereas it was approximately 40 times less potent as a bronchodilator (Table 1).

Previous in vitro studies using guinea-pig tracheal preparations (Raper et al 1978; Iakovidis et al 1979) have shown that RO363 can unmask pharmacological activity due to the stimulation of subpopulations of β_1-adrenoceptors in tissues where β_2-receptors represent the dominant receptor type. Lulich et al (1976) have presented evidence which suggests that β_1- as well as β_2-adrenoceptors are present in the larger airways of the cat, and thus it is possible that the intermediate potency of RO363 as a bronchodilator reflects its ability to stimulate this mixed β_1- and β_2-receptor population.

Preliminary experiments in rabbits suggest that β_1-receptor stimulation may also be involved in the vasodepressor responses to RO363. In these studies responses to equipotent bolus doses of sympathomimetic amines were monitored in the absence and in the presence of increasing cumulative doses of the β_1-adrenoceptor selective antagonist atenolol (Barrett et al 1973) administered intravenously at 15 min intervals. Doses of atenolol causing a 50% inhibition of agonist responses (ID50 values) were calculated from the antagonist dose-inhibition curves. Positive chronotropic responses to both (−)-isoprenaline and RO363 were antagonized over a similar atenolol dose-range (ID50, 0·09–0·19 mg kg^{-1}; n = 4). As expected, larger doses of atenolol were required to antagonize the β-receptor mediated vasodepressor responses to (−)-isoprenaline and salbutamol (ID50, 2·4–4·0 mg kg^{-1}; n = 4). However, the vasodepressor responses to RO363 were inhibited over an intermediate atenolol dose-range (ID50, 0·4–0·8 mg kg^{-1}; n = 4). These results are compatible with the involvement of β_1-adrenoceptors in the vasodepressor response to RO363. In future experiments it is intended to investigate the effects of RO363 in a variety of vascular beds in an attempt to determine the site of this β_1-receptor mediated vasodilator activity.

In conclusion, the results of the present in vivo experiments substantiate in vitro findings and show that RO363 is a potent and highly selective β_1-adrenoceptor agonist. The results also highlight potential difficulties in assessing the selectivity of drugs in situations where mixed receptor populations might be involved.

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