Br. J. Pharmac. (1973), 47, 398–407.

Selective blockade of β-adrenoceptors by 1-(*p*-allylphenoxy)-3-isopropylamino-2 propanol hydrochloride (H 64/52) in the anaesthetized dog

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Summary

1. The β-adrenoceptor blocking properties of 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride (H 64/52) on the cardiovascular and bronchomotor responses to isoprenaline have been determined in the anaesthetized dog.

2. H 64/52 selectively blocked the isoprenaline-induced increase in heart rate, contractile force and reduction in airway pressure in the same dose range.

3. H 64/52 produced a minimal blockade of vascular β-adrenoceptors which was apparent only when the isoprenaline-induced fall in diastolic pressure was considered. No significant vascular β-adrenoceptor blockade with H 64/52 could be shown with the femoral arterial flow or hind-limb perfusion studies.

4. These results further support the hypothesis that several different β-adrenoceptor subtypes exist in the dog. The β-adrenoceptors subserving cardiac stimulation and bronchodilation appear to be similar and both differ from the β-adrenoceptor subserving vasodilatation.

Introduction

It has been suggested that β-adrenoceptor antagonists may be classified into at least three types (Levy & Wilkenfeld, 1968). One type is capable of blocking vascular β-adrenoceptors relatively more than myocardial β-adrenoceptors and includes butoxamine (Levy, 1966) as a prototype. The second type is capable of blocking myocardial β-adrenoceptors relatively more than vascular β-adrenoceptors and includes practolol (Dunlop & Shanks, 1968) as a prototype. The third type is capable of blocking β-adrenoceptors in all tissues in the same dose range and includes the 'classical' β-adrenoceptor antagonists dichloroisoprenaline, pronethalol, sotalol, alprenolol and propranolol.

The purpose of this study is to demonstrate the selective β-adrenoceptor antagonist properties of 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride (H 64/52). The results indicate that H 64/52 exerts β-adrenoceptor blocking properties that are cardiac and bronchiolar selective and are qualitatively similar to those of practolol.

Methods

Adult mongrel dogs of either sex, weighing 10–20 kg were anaesthetized with a combination of barbital sodium (220 mg/kg) and pentobarbital sodium (20 mg/kg) given together intravenously. All dogs were bilaterally vagotomized.

Cardiovascular studies

One group of 8 dogs was used to determine the effects of isoprenaline on diastolic blood pressure, myocardial contractile force, heart rate and femoral arterial blood flow before and after treatment with H 64/52. Arterial blood pressure was recorded in mmHg (1 mmHg≡1·333 mbar) from a carotid artery by means of a Statham transducer. Heart rate was recorded continuously with a linear electronic tachometer, triggered by the arterial pulse. Cardiac contractile force was measured with animals under positive pressure artificial respiration. The heart was exposed by a thoracotomy and a calibrated strain-gauge arch was sutured to the right ventricle. The dogs were respired with a tidal volume of approximately 13 ml/kg of air at a frequency of 20 per minute. Femoral arterial blood flow was recorded with a square-wave electromagnetic flowmeter (Model 301, Carolina Medical Electronics); non-cannulating flow probes with a circumference of 5, 7 or 10 mm were used, depending on the size of the vessel. In the blood flow studies, isoprenaline was injected intra-arterially into a small branch of the femoral artery so that arterial flow was not interrupted. All other injections of isoprenaline and H 64/52 were made into the right jugular vein. Two intravenous doses (0·3 and 1·0 μg/kg) and one intra-arterial dose (0·03 μg/kg) of isoprenaline were injected before and after cumulative, intravenous doses of 0·03, 0·1, 0·3, 1·0 and 3·0 mg/kg of H 64/52.

In a second group of 6 dogs arterial blood pressure, heart rate and myocardial contractile force were recorded as described above. In this group, dose-response curves for isoprenaline were determined before and after cumulative doses of 0·3, 1·0 and 3·0 mg/kg of H 64/52. All drugs in this group were injected intravenously.

The effect of isoprenaline on femoral arterial perfusion pressure, before and after H 64/52 treatment, was determined in a third group of 6 dogs. The right femoral artery was cannulated and perfused with arterial blood taken from the cannulated left femoral artery at a constant rate by means of a peristaltic pump (Harvard Apparatus Co., Model 1215). In this group of dogs only, the blood was rendered incoagulable by the intravenous injection of 5 mg/kg of heparin initially, followed by 2·5 mg/kg every subsequent half hour. Systemic arterial pressure and femoral arterial perfusion pressure were measured continuously with Statham pressure transducers (P 23AA) on the inflow and outflow side of the peristaltic pump respectively (Nakano & McCloy, 1967). Dose-response curves for isoprenaline were obtained by intra-arterial injection through rubber tubing on the outflow side of the peristaltic pump before and after H 64/52 treatment. Cumulative doses of 0·1, 0·3, 1·0 and 3·0 mg/kg H 64/52 were administered intravenously.

Airway pressure studies

Changes in intra-tracheal pressure (airway pressure) were recorded in a group of 6 dogs by the technique described by Hinds & Katz (1971). Airway pressure changes were recorded by means of a Statham pressure transducer (P 23-BB) placed in the ventilatory circuit. The animals were artificially respired as described above. Gallamine triethiodide, 6 (mg/kg)/h, was infused continuously to prevent spontaneous respiration. Neostigmine methylsulphate, 0·2 (mg/kg)/h, was also infused continuously to maintain a sustained increase in airway pressure (Dungan, Cho, Gomoll, Aviado & Lish, 1968). Neostigmine and gallamine were both infused into a femoral vein. Isoprenaline, in a standard dose of 0·3 μg/kg, was injected intravenously before and after treatment with H 64/52 in cumlative doses of

0·03, 0·1, 0·3, 1·0 and 3·0 mg/kg. Isoprenaline and H 64/52 were injected into the right jugular vein.

In all studies each dose of H 64/52 was allowed to act for at least 10 min before the injection of isoprenaline. Consecutive doses of isoprenaline were administered at 5 min intervals. (±)-Isoprenaline hydrochloride was prepared as a 10^{-3} g/ml stock solution in distilled water. A 10^{-4} dilution was prepared fresh daily for each experiment. Doses of isoprenaline are in terms of the base. 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride (H 64/52) was prepared as a 1% solution in distilled water. Doses of H 64/52 are in terms of the salt.

Results

Effect of H 64/52 on the cardiovascular responses to isoprenaline

The effects of H 64/52 treatment on the vardiovascular responses to isoprenaline were determined in a total of 20 dogs. These cardiovascular experiments were divided into three groups.

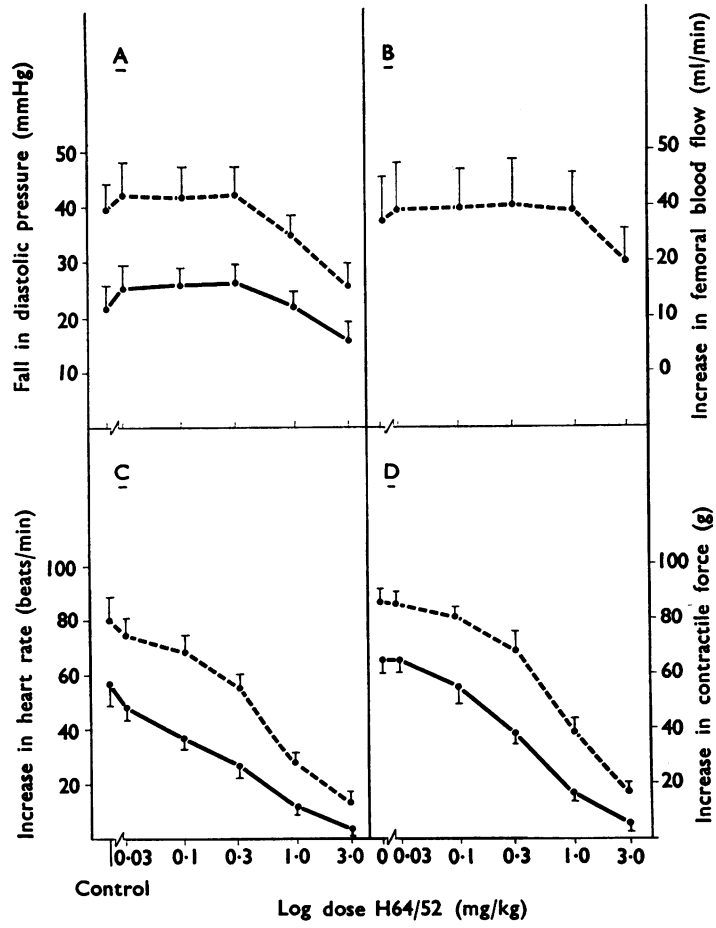

FIG. 1. Effects of H 64/52 treatment on the fall in diastolic pressure (A), the increase in femoral arterial bloodflow (B), positive chronotropic (C) and positive inotropic (D) responses to isoprenaline. Doses of isoprenaline in A, C and D: ●——● (0·3 μg/kg, i.v.), ●- - -● (1·0 μg/k i.v.). Dose of isoprenaline in B only ●- - -● (0·03 μg/kg, i.a.). Each point represents the mean ± S.E.M. of 8 dogs.

Group 1 (8 dogs)

Two control doses of isoprenaline, 0·3 and 1·0 μg/kg, were injected i.v. before and after treatment with H 64/52 in a cumulative dose range of 0·03–3·0 mg/kg. An additional dose of 0·03 μg/kg of isoprenaline was injected into the femoral artery before and after H 64/52 treatment. Arterial blood pressure, heart rate, myocardial contractile force and femoral arterial blood flow were recorded. The results obtained with this group are shown in Figure 1. H 64/52, after a cumulative dose of 0·1 mg/kg, produced a significant reduction in the positive inotropic (Fig. 1D) and the positive chronotropic (Fig. 1C) responses to both doses of isoprenaline. The isoprenaline-induced fall in diastolic pressure (Fig. 1A) and the increase in femoral blood flow (Fig. 1B) were significantly reduced only after 3 mg/kg of H 64/52. It was shown previously (Levy, 1972) that the control responses to the three doses of isoprenaline used in this study were submaximal responses. The intrinsic effects of H 64/52 on the cardiovascular system were determined in this group of dogs by comparing each of the measurements before and 10 min after H 64/52 treatment, prior to the injection of isoprenaline. These results are summarized in Figure 2. H 64/52 treatment resulted in a significant degree of

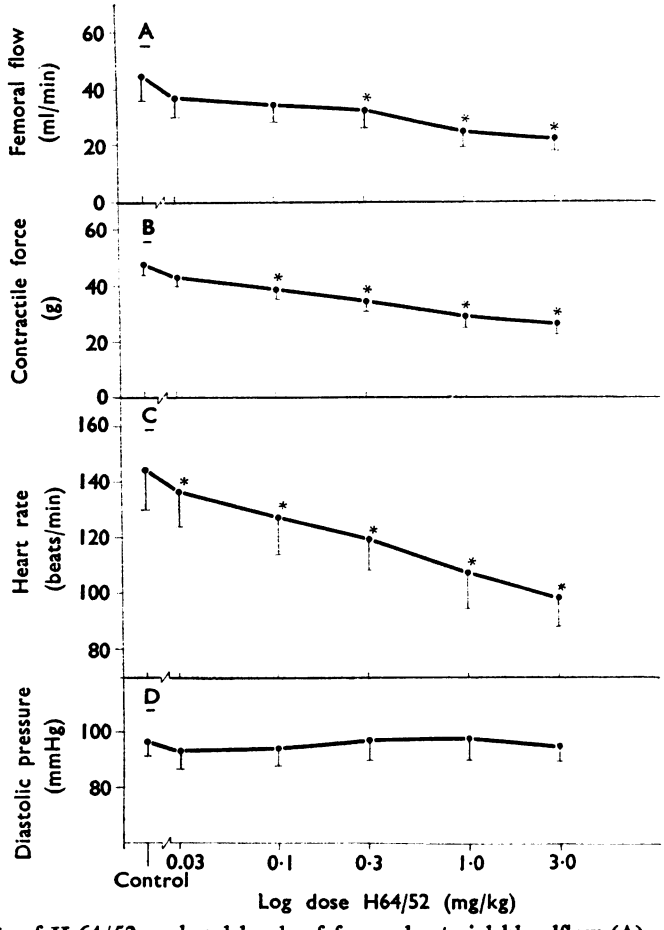

FIG. 2. Effects of H 64/52 on basal levels of femoral arterial bloodflow (A), myocardial contractile force (B), heart rate (C) and diastolic pressure (D) in anaesthetized dogs. Each point represents the mean ± S.E.M. from 8 dogs. Asterisk indicates significant effect ($P<0·05$).

bradycardia (Fig. 2C) after the initial dose of 0·03 mg/kg. Myocardial contractile force was significantly reduced after 0·1 mg/kg (Fig. 2B), femoral arterial blood flow was reduced after 0·3 mg/kg (Fig. 2A) but diastolic blood pressure was not reduced significantly even after 3 mg/kg of H 64/52 (Fig. 2D). The ability of H 64/52 to produce a degree of cardiac depression is a property shared by most β-adrenoceptor antagonists whether they are selective or non-selective in action.

Group 2 (6 dogs)

Arterial blood pressure, heart rate and contractile force were recorded in this group of animals. All drugs were given intravenously. Dose-response curves for isoprenaline were determined before and after cumulative doses of 0·3, 0·1 and 3·0 mg/kg of H 64/52. The purpose of these experiments was to demonstrate whether or not H 64/52 was exerting a β-adrenoceptor blocking effect that was competitive and reversible. Figures 3B and 3C show that treatment with H 64/52 resulted in a parallel shift to the right of the isoprenaline dose-response curves obtained for heart rate and myocardial contractile force. This shift was quite

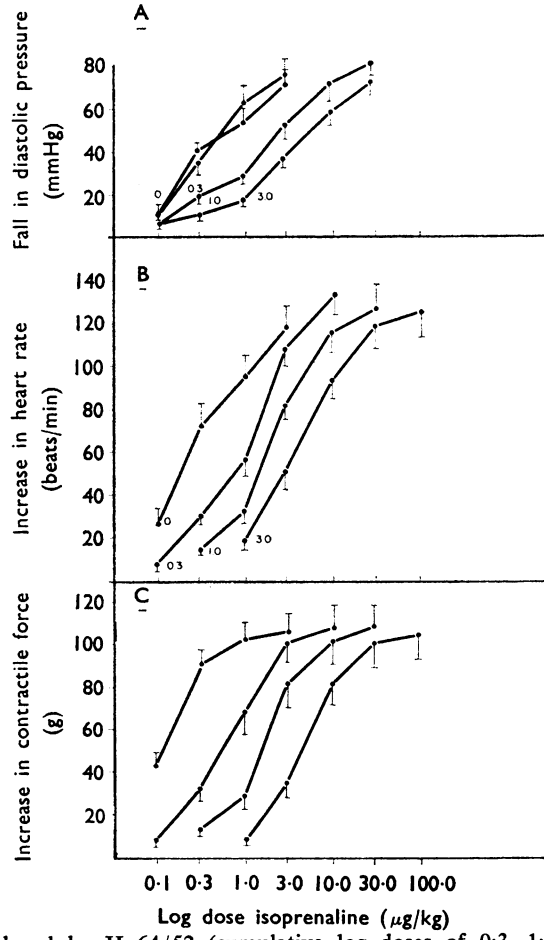

FIG. 3. Shifts produced by H 64/52 (cumulative log doses of 0·3, 1·0 and 3·0 mg/kg as indicated) in the dose-response curves of the fall in diastolic pressure in mmHg (A), increase in heart rate in beats/min (B) and the increase in contractile force in g (C) induced by intravenous isoprenaline in anaesthetized dogs. Each curve represents the mean ±S.E.M. of 6 dogs. Abscissae in all panels: log dose of isoprenaline in μg/kg.

marked with the smallest dose of H 64/52 given, 0·3 mg/kg. Figure 3A shows that H 64/52 treatment resulted in some shift of the isoprenaline dose-response curve for the reduction in diastolic blood pressure. This shift was not significant until after a cumulative dose of 1·0 mg/kg of H 64/52 had been given. There was also no depression of the maximal response to isoprenaline on this parameter. These results would suggest that H 64/52 is exerting a reversible competitive blocking effect of the β-adrenoceptors responsible for the isoprenaline-induced fall in diastolic pressure as well as those β-adrenoceptors responsible for the positive inotropic and chronotropic responses to isoprenaline. However, H 64/52 appears to be exerting a quantitatively weaker β-adrenoceptor blocking effect on the vasculature than in the myocardium.

Group 3 (6 dogs)

The effect of H 64/52 treatment on the isoprenaline-induced reduction in femoral arterial perfusion pressure was determined by giving isoprenaline, in a dose range of 0·01 to 0·3 μg/kg, intra-arterially into the femoral vascular bed by means of a small segment of rubber tubing on the outflow side of the peristaltic pump. This dose range of isoprenaline was repeated before and after the intravenous injection of cumulative doses of 0·1, 0·3, 1·0 and 3·0 mg/kg of H 64/52. The effects on femoral arterial perfusion pressure reflects the activation of vascular β-adrenoceptors by isoprenaline more accurately than do changes in diastolic or mean arterial pressure. The results obtained in this group of dogs closely resemble those obtained with the femoral arterial bloodflow studies. Figure 4C shows that the isoprenaline dose-response curve was not shifted significantly even after the maximum dose of 3 mg/kg of H 64/52.

The technique of femoral arterial perfusion which was used (Nakano & McCloy, 1967) involved adjusting the pump speed initially so that the perfusion pressure corresponded to the systemic arterial pressure and was subsequently unaltered. Figure 4B shows that the femoral arterial perfusion pressure was significantly elevated above initial control limits after a cumulative dose of 0·3 mg/kg of H 64/52. The systemic arterial blood pressure did not differ significantly from the initial control levels even after 3 mg/kg of H 64/52 (Fig. 4A). The elevated femoral arterial perfusion pressure represents a peripheral vasoconstrictor effect that is characteristic of this experimental technique. We have found that the femoral arterial perfusion pressure gradually increases in other studies which involved the use of the β-adrenoceptor antagonists propranolol, practolol, and butoxamine as well as the β-adrenoceptor agonists isoprenaline, terbutaline and salbutamol (Wasserman & Levy, 1972). Resting femoral arterial pressure also gradually increases even when no drugs are given.

Effects of H 64/52 on the isoprenaline-induced reduction in airway pressure

Isoprenaline produced a significant reduction in airway pressure in dogs treated with neostigmine to increase airway pressure. This response to isoprenaline may be considered as an index of its bronchodilator activity. H 64/52 reduced this isoprenaline bronchodilator response in a group of 6 dogs. Figure 5 shows that H 64/52 had a significant blocking effect on bronchial β-adrenoceptors which was dose-dependent and could be seen after a dose of 0·03 mg/kg. The level of bronchoconstriction or increased airway pressure produced by neostigmine infusion was constant throughout all experiments.

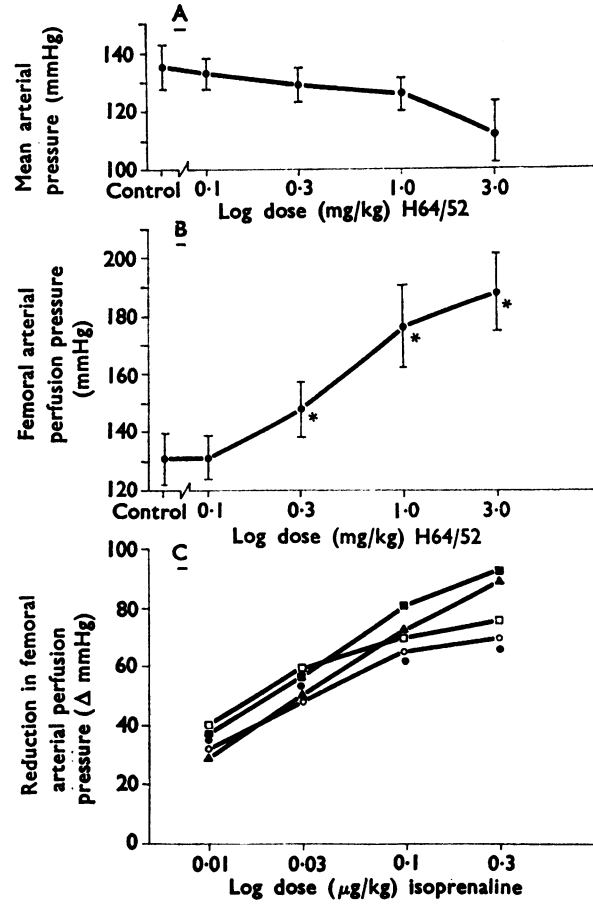

FIG. 4. Effects of H 64/52 on mean arterial pressure (A), femoral arterial perfusion pressure (B) and the reduction in femoral arterial perfusion pressure by isoprenaline (C). Isoprenaline dose-response curves before and after H 64/52 treatment are shown in C. ●, Control; ○, H 64/52 0·1 mg/kg; □, H 64/52 0·3 mg/kg; ■, H 64/52 1·0 mg/kg; ▲, H 64/52 3·0 mg/kg. Each point represents mean ± S.E.M. of 6 dogs. Asterisk indicates significant difference from initial control ($P<0.05$).

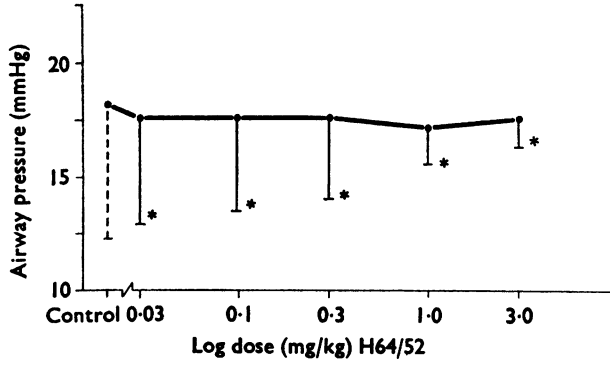

FIG. 5. Effects of H 64/52 treatment on the isoprenaline-induced reduction in airway pressure in the anaesthetized dog. Top connected tracing indicates resting airway pressure. Bars indicate maximum responses after isoprenaline (0·3 μg/kg, i.v.). Each line represents the mean obtained from 6 dogs. Asterisk indicates significant differences of isoprenaline-induced fall in airway pressure from initial control response ($P<0.05$).

Results obtained with all three groups of animals suggests that H 64/52 exerts a more selective β-adrenoceptor blocking effect on cardiac and bronchial β-adrenoceptors than it does on vascular β-adrenoceptors.

Discussion

Lands, Luduena & Buzzo (1967) have proposed that β-adrenoceptors can be divided into β-1 and β-2 subtypes, based upon composite results obtained from several different animal species and from *in vitro* and *in vivo* studies. Since it is more satisfactory if the classification of an agent as a selective β-adrenoceptor agonist or antagonist is based upon results obtained from the same species and under similar experimental conditions, this report describes the relative ability of H 64/52 to antagonize β-adrenoceptors in the heart, the vasculature and bronchial smooth muscle in the anaesthetized dog.

H 64/52 produced a competitive and reversible blockade of the myocardial β-adrenoceptors as shown by the reduction in both the positive inotropic and chronotropic responses to isoprenaline (Figs. 1C, 1D, 3B and 3C). Doses 30 times larger were needed to produce equivalent blockade of the vasodepressor responses to isoprenaline (Fig. 1). However, changes in arterial blood pressure may be influenced by factors other than simple direct effects on vascular smooth muscle and studies utilizing femoral arterial blood flow and the perfused hind limb reflect more accurately the actions of isoprenaline on β-adrenoceptors in vascular smooth muscle. Results obtained with both methods (Figs. 1B and 4) confirm that H 64/52 exerts a relatively weak competitive blocking action on certain vascular β-adrenoceptors although no significant vascular effect could be demonstrated following a cumulative dose of 3·0 mg/kg. A comparison of isoprenaline dose-response shifts demonstrated similar, qualitative, selective differences in the β-adrenoceptor blocking properties of H 64/52. Figures 3B and 3C show that H 64/52, after the initial dose of 0·3 mg/kg and all subsequent doses, produced a parallel shifting to the right of the isoprenaline dose-response curves determined for the myocardial response. Figure 3A indicates that H 64/52 also shifted the isoprenaline dose-response curve for its diastolic blood pressure responses to the right but to a significantly lesser extent. Boissier, Advenier, Guidicelli & Viars (1971) reported a similar parallel shift for the isoprenaline-induced reductions in diastolic blood pressure by proctolol. Their results indicated that this shift was not dose-related, was pronounced at low doses and quickly reached a plateau. The selectivity is thus relative and not absolute, and a certain amount of overlapping of β-adrenoceptor blocking effects occurs with all antagonists on all β-adrenoceptors.

H 64/52 also reduced the bronchodilator response to 0·3 μg/kg of isoprenaline with doses which were roughly equivalent to those needed to block the isoprenaline-induced positive inotropic and chronotropic responses.

H 64/52, in the anaesthetized dog, exerts a more selective β-adrenoceptor blocking effect on the heart and bronchiolar smooth muscle than it does on vascular smooth muscle. These results confirm and extend the observations of Ablad, Brogard, Carlsson & Ek (1970). Unlike its ortho and meta (alprenolol) analogue, H 64/52 was a cardiac selective β-adrenoceptor blocking agent in the anaesthetized cat. Since H 64/52 differs from its two analogues in that it is a para-allyl derivative rather than an ortho or meta derivative, it would seem that the para substitution may be responsible for the selective β-adrenoceptor properties of H 64/52. This

conclusion would appear to be strengthened by the fact that practolol, another cardiac selective β-adrenoceptor antagonist, is also a para substituted compound. However, sotalol (MJ 1999) and INPEA are two examples of non-selective β-adrenoceptor antagonists which are also para substituted compounds. It is obvious that para substitution alone does not necessarily impart selective β-adrenoceptor blocking activity to a given compound.

H 64/52 qualitatively and quantitatively resembles practolol in its β-adrenoceptor blocking activity. Both compounds produce nearly complete blockade of cardiac β-adrenoceptors with doses which exert little effect on vascular β-adrenoceptors. H 64/52, like practolol, also has a significant β-adrenoceptor blocking effect on bronchiolar smooth muscle as well as the myocardium. Practolol has a pA_2 of 7·27 against isoprenaline on isolated guinea-pig trachea (Levy & Wilkenfeld, 1970) and in the guinea-pig atria has a pA_2 of 7·3 (Farmer & Levy, 1970). Boissier *et al.* (1971) were able to show that practolol produced potent β-adrenoceptor blocking effects in the heart as well as the bronchiolar musculature in anaesthetized dogs. Finally, practolol like propranolol, has been reported to produce an asthmatic attack in man (Bonn, Turner & Hicks, 1972) indicating a significant β-adrenoceptor blocking effect on bronchiolar smooth muscle in man. The classification of the β-adrenoceptors in cardiac muscle and vascular smooth muscle as two distinct β-adrenoceptor subtypes is well supported by experimental evidence (Levy, 1966; Levy & Wilkenfeld, 1968; Dunlop & Shanks, 1968). However, the classification of bronchial and vascular β-adrenoceptors into the same category (β-2) as proposed by Lands, Luduena & Buzzo (1967), is not well supported by experimental evidence if one considers comparative results obtained from the same species. Although the concept of several β-adrenoceptor subtypes is acceptable the present classification of β-1 and β-2 adrenoceptor subtypes seems unnecessarily restrictive and considerably oversimplified.

This project was supported by USPHS Grant HE-12989. We wish to thank Dr. B. Ablad (A. B. Hassle, Goteborg, Sweden) for the generous supply of H/64/52. The technical assistance of Margaret E. Frazer is gratefully acknowledged.

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(*Received June* 27, 1972)