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1-Isopropylamino-3-(4-indanoxy)-2-propanol HCl: a potent β-adrenoceptor antagonist

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Summary

1. The β-adrenoceptor blocking activity of 1-isopropylamino-3-(4-indanoxy)-2-propanol HCl (USVC 6524) was determined in the anaesthetized dog, the isolated rat uterus and the isolated guinea-pig tracheal strip.

2. USVC 6524 inhibited the positive inotropic, positive chronotropic and vasodilator responses to isoprenaline in the dog in a dose of 10 μg/kg and higher. On the basis of comparative pA_2 values, USVC 6524 is approximately 10 times more potent as a β-adrenoceptor antagonist than propranolol.

3. Cardiac depressant effects produced by USVC 6524 were relatively mild and occurred only after the onset of a strong β-adrenoceptor blockade.

4. USVC 6524 also blocked β-adrenoceptors in the isolated rat uterus and guinea-pig tracheal spiral strip.

Introduction

The presently accepted classification of adrenoceptors into α and β types was originally conceived by Ahlquist (1948) and was based experimentally on differences in the orders of responsiveness to a series of catecholamines closely related to and including adrenaline. Further proof in support of this receptor classification was the observation of the ability of dichloroisoprenaline (DCl) to block β-adrenoceptors selectively (Powell & Slater, 1958). Since the discovery of DCl, numerous other β-adrenoceptor antagonists have been described, including pronethalol (Black & Stephenson, 1962), MJ-1999 (Stanton, Kirchgessner & Parmenter, 1965) and propranolol (Black & Stephenson, 1962). Propranolol has recently been introduced into therapeutics principally for the treatment of angina pectoris as well as a variety of cardiac arrhythmias.

The purpose of this study was to determine the β-adrenoceptor blocking properties of 1-isopropylamino-3-(4-indanoxy)-2-propanol HCl (USVC 6524). This agent differs structurally from propranolol in that it possesses an indane rather than a

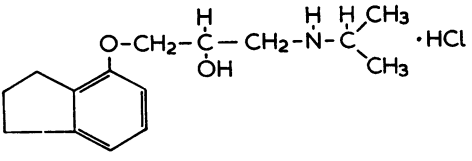

FIG. 1. Structural formula of 1-isopropylamino-3-(4-indanoxy)-2-propanol HCl (USVC 6524).

naphthyl group. Results obtained in this study show this compound to be a β-adrenoceptor antagonist of considerable potency with relatively weak cardiac depressant properties. The structure of USVC 6524 is given in Fig. 1.

Methods

Anaesthetized dogs

Sixteen 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 used were bilaterally vagotomized. Mean arterial pressure was recorded in mmHg (1 mmHg$\equiv$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 strain-gauge arch was sutured to the right ventricle. 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. Drugs were injected intra-arterially into a small branch of the femoral artery that was cannulated with a fine polyethylene cannula (PE-10). In this manner femoral arterial flow was not interrupted. All other drug injections were made into the right external jugular vein. Recordings were made with a multichannel cathode-ray tube camera system (Model DR-8, Electronics for Medicine).

Two groups of five dogs were used to determine the β-adrenoceptor blocking action of USVC 6524. In one group mean arterial pressure, heart rate and myocardial contractile force were recorded in open chest dogs. Dose-response curves for isoprenaline were determined before and after treatment with increasing doses of antagonist. The initial control doses for intravenous isoprenaline were 0·1, 0·3 and 1 μg/kg. The antagonist was administered in doses of 3, 10, 30, 100 and 1,000 μg/kg intravenously. Maximum responses to isoprenaline were determined after each dose of USVC 6524 by increasing the doses of isoprenaline logarithmically as β-adrenoceptor blockade occurred. The total dose range for isoprenaline in this group of dogs was 0·1–300 μg/kg. All doses of isoprenaline were given at 5 min intervals. Each dose of antagonist was allowed to act for 10 min before determining the isoprenaline dose-response curve. All dogs in all studies were allowed a 30 min equilibration period.

In the second group of five dogs mean arterial pressure, heart rate and femoral blood flow were recorded. The doses of the antagonist were the same as those in the first group and were also given intravenously. Isoprenaline was only injected intra-arterially. The initial control doses for isoprenaline were 0·001, 0·003, and 0·01 μg/kg. Following treatment with USVC 6524 the doses of isoprenaline were increased in order to obtain a response approximately equal to the initial control isoprenaline responses. In this group a dose range for isoprenaline of 0·001–30 μg/kg was used. Quantitative evaluation for isoprenaline antagonism in both groups by USVC 6524 was performed by determination of pA_2 according to the method of Arunlakshana & Schild (1959).

A third group of six dogs was used to determine the effects of USVC 6524 alone on mean arterial pressure, heart rate and myocardial contractile force. USVC 6524 was given in doses of 3, 10, 30, 100, 300 and 1,000 μg/kg at 15 min intervals. The effects of USVC 6524 itself were measured 10 min after each dose. The statistical significance of the differences between means was determined by the *t* test for paired data.

Isolated tissues

Rat uterus

Female rats, weighing 140–160 g, were injected subcutaneously with 0·1 mg/kg of stilboestrol and killed 24 h later. Uterine segments were suspended in 10 ml organ baths at a constant temperature of 38° C in Locke's solution (g/100 ml: NaCl 0·9, KCl 0·042, $CaCl_2$ 0·024, glucose 0·1 and $NaHCO_3$ 0·05), bubbled with 95% oxygen and 5% carbon dioxide. Spontaneous uterine contractions soon appeared. Uterine segments were also suspended in de Jalon's solution (g/100 ml: NaCl 0·9, KCl 0·042, $CaCl_2$ 0·006, glucose 0·05 and $NaHCO_3$ 0·05) at 25° C. Submaximal uterine contractions were evoked by exposure to 10^{-6}M acetylcholine acting for 30 s at 3 min intervals. Concentration-response curves for isoprenaline were determined by allowing molar concentrations of isoprenaline to act for 3 min before challenge with acetylcholine. Inhibitory responses to isoprenaline are expressed as % reduction of the control acetylcholine-induced contraction. Molar concentrations of USVC 6524 were allowed to act for 60 min before determining concentration-response curves for isoprenaline. Antagonist potency in this preparation was measured by pA_2 determinations (Arunlakshana & Schild, 1959).

Guinea-pig trachea

Guinea-pigs of either sex weighing 400–500 g were used. The guinea-pigs were killed by a blow on the head, and the tracheas were removed and carefully cleaned of connective tissue. A wooden applicator stick was inserted into the trachea and fixed with a pin at one end. The trachea was next cut with a sharp scalpel blade in a spiral fashion to produce a thin strip of tracheal tissue (Patterson, 1958). The tracheal spiral from a single guinea-pig was then cut into two equal segments and both were then placed in 10 ml baths of Krebs-Henseleit solution (g/100 ml: NaCl 0·69, KCl 0·0353, $CaCl_2$ 0·028, $MgSO_4 \cdot H_2O$ 0·0294, $NaHCO_3$ 0·21, KH_2PO_4 0·0165 and glucose 0·2). The bath fluid also contained ascorbic acid in a concentration of 10^{-4} g/ml. Temperature was maintained at 38° C and the solution was gassed with 95% oxygen and 5% carbon dioxide. An initial basal tension of 2 g was applied to each tracheal strip and the tissue was allowed to stand for 1 h before use. A constant level of tone was induced by exposure to a concentration of $5{\cdot}5 \times 10^{-7}$M of carbachol. The carbachol was allowed to act for 15 min. Following this and in the constant presence of carbachol, cumulative dose-response curves to isoprenaline were determined before and after treatment with USVC 6524. Each dose of isoprenaline was allowed to act until the response was stabilized before the next cumulative dose was added ; this usually required 3 to 5 min. After obtaining a control concentration-response curve for isoprenaline, the tissues were washed thoroughly and 5 min later a concentration of USVC 6524 was added. The USVC 6524 was allowed to act for 60 min. Two concentrations of USVC 6524 were used

in each tracheal strip and the doses were increased ten-fold each time. The pA_2 value was calculated in the same manner as described previously for the rat uterus as a measurement of the potency of the β-adrenoceptor antagonism produced by USVC 6524 in this preparation. Isometric contractions were measured in both rat uterus and guinea-pig trachea by means of a force displacement transducer (Grass Ft. 03) connected to a polygraph (Grass Model 5D).

Drugs

(±)-Isoprenaline hydrochloride was prepared as a 10^{-3} g/ml stock solution for the dog experiments and a 10^{-2}M stock solution for the isolated tissue studies. These dilutions were made in 0·9% NaCl solution containing 10^{-4} g/ml ascorbic acid; working dilutions of these stock solutions were prepared fresh daily. 1-Isopropylamino-3-(4-indanoxy)-2-propanolol HCl (USVC 6524) was prepared as a 0·5% solution in 0·9% NaCl solution. Acetylcholine was prepared as a 10^{-2}M stock solution and carbachol chloride was prepared as a $5{\cdot}5 \times 10^{-5}$M stock solution.

Results

Effects of USVC 6524 in the anaesthetized dog

The effects of USVC 6524 treatment in a dose range of 3–1,000 μg/kg on the cardiovascular responses to isoprenaline were determined in two groups of five dogs. Since we were determining dose-response relationships for isoprenaline given intravenously and intra-arterially, two groups of dogs were used so that the control periods between doses of USVC 6524 would not be excessively long. Dose-response curves for the effects of intravenous isoprenaline on heart rate and myocardial contractile force were determined in one group before and after USVC 6524 treatment.

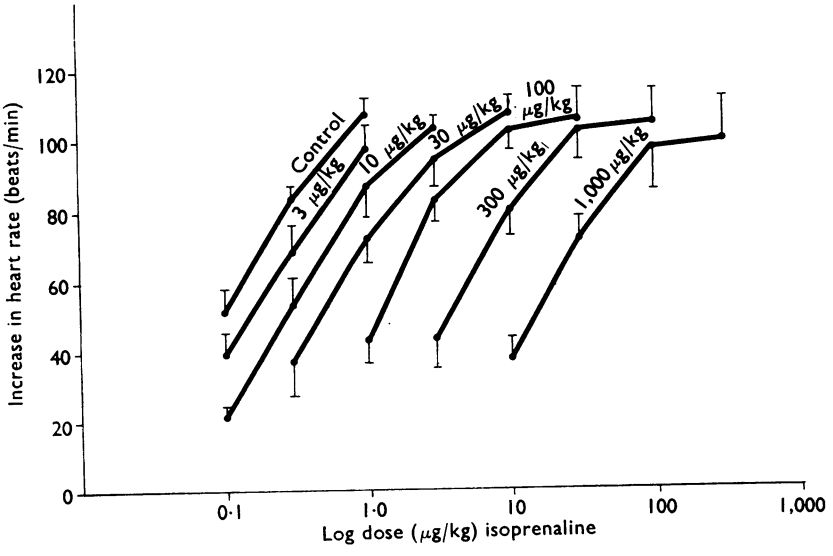

FIG. 2. Shift produced by compound USVC 6524 (3-1,000 μg/kg, as indicated) in the dose-response curve of the positive chronotropic effect induced by intravenous isoprenaline in anaesthetized dogs. Each curve represents the mean ± standard error of five dogs. Abscissa: log dose of isoprenaline in μg/kg. Ordinate: increase in heart rate in beats/min over pre-injection control rate.

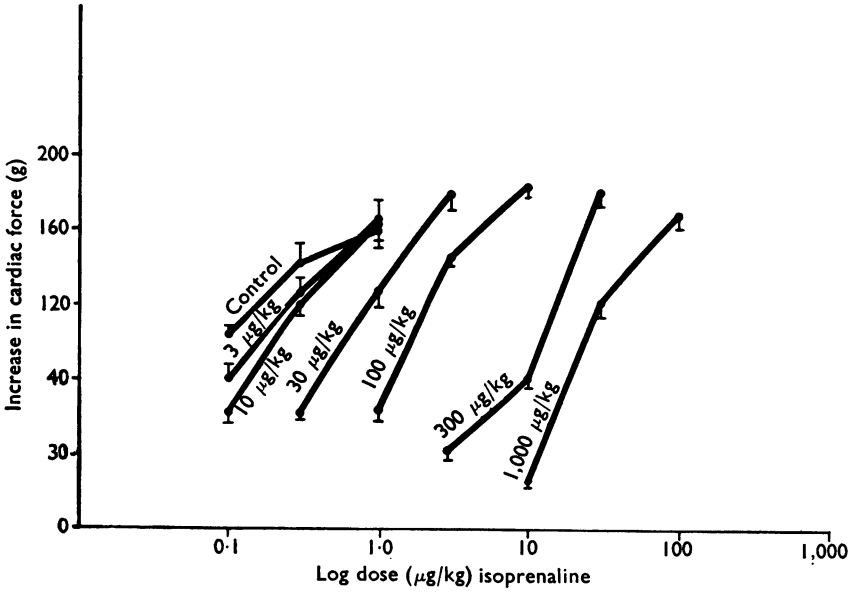

FIG. 3. Shift produced by compound USVC 6524 (3-1,000 μg/kg, as indicated) in the dose-response curve for the positive inotropic effect induced by intravenous isoprenaline in anaesthetized dogs. Each curve represents the mean ± standard error of five dogs. Abscissa: log dose of isoprenaline in μg/kg. Ordinate: increase in contractile force in grams over the pre-injection control value.

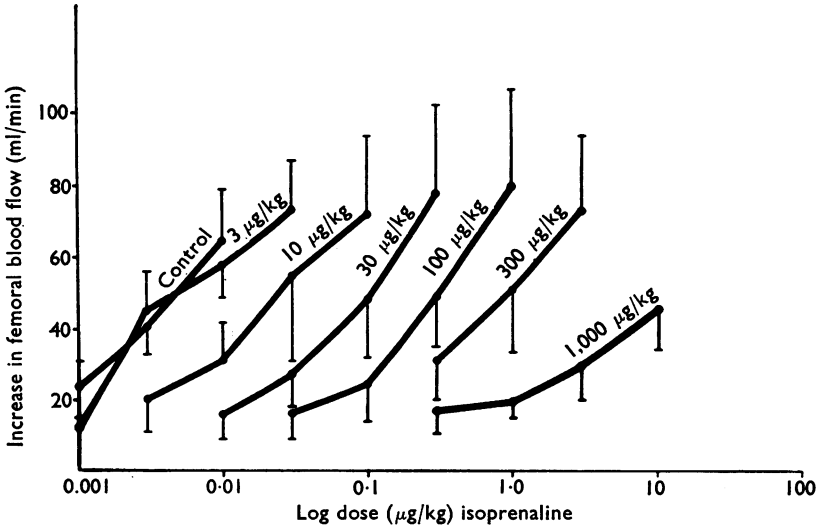

FIG. 4. Shift produced by compound USVC 6524 (3-1,000 μg/kg, as indicated) in the dose-response curve for the increase in femoral arterial blood flow induced by intra-arterial isoprenaline in anaesthetized dogs. Each curve represents the mean ± standard error of five dogs. Abscissa: log dose of isoprenaline in μg/kg. Ordinate: increase in blood flow in ml/min over the pre-injection control value.

Dose-response curves for the effects of intra-arterial isoprenaline on blood flow were determined in the second group of dogs before and after treatment with USVC 6524. USVC 6524 induced a parallel shift to the right of the isoprenaline dose-response curves for all three parameters. Figures 2 and 3 demonstrate the ability of USVC 6524 to shift significantly the isoprenaline dose-response curves for heart rate and myocardial contractile force, respectively ; β-adrenoceptor blockade by USVC 6524 is evident here after doses of 3–10 μg/kg. Similar effects on femoral blood flow response to intra-arterial isoprenaline were also produced by USVC 6524. Figure 4 demonstrates the ability of USVC 6524 to shift the dose-response curve for isoprenaline on femoral blood-flow to the right. Again, a potent β-adrenoceptor blocking effect is produced by a dose of 10 μg/kg of USVC 6524. Increasing the dose of USVC 6524 resulted in a marked increase in the doses of isoprenaline needed to produce the same approximate maximal responses as the initial control values. No attempt was made to draw dose-response curves for the effects of isoprenaline on mean arterial pressure because of the ability of USVC 6524 to convert the isoprenaline depressor response to a pressor response, particularly with the lower doses of isoprenaline. Figure 5 summarizes the effects of USVC 6524 on the three cardiovascular parameters cited ; log dose of USVC 6524 in mol/kg is plotted against log (dose ratio−1). Regression lines of USVC 6524 were calculated by the method of least squares and each curve represents the pooled results obtained from five dogs. Regression lines were calculated using the doses of 10, 30, 100, 300, and 1,000 μg/kg of USVC 6524 because significant β-adrenoceptor blockade did not occur until after a dose of 10 μg/kg had been given. The dose ratios were determined at the approximate mid-point of the isoprenaline dose-response curves and

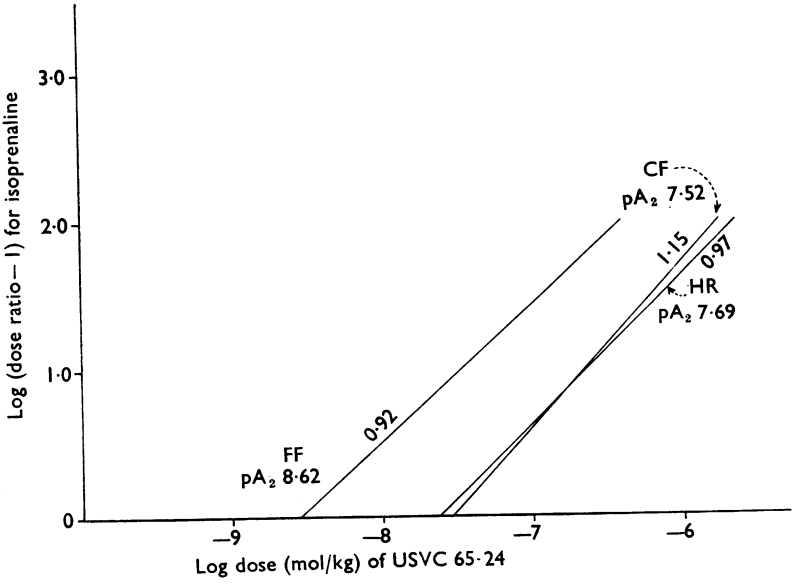

FIG. 5. Regression lines of USVC 6524 for isoprenaline antagonism on heart rate (HR), contractile force (CF) and femoral arterial blood-flow (FF) in the anaesthetized dog. Lines were calculated by the method of least squares from the pooled results of five dogs. For each concentration of USVC 6524 the ratio of the equiactive dose of isoprenaline in the presence and absence of antagonist (dose ratio) was calculated. Log (dose ratio − 1) was plotted against log dose of USVC 6524 in mol/kg. Numbers beside regression lines indicate slope and pA_2.

are the ratios of isoprenaline doses producing equal responses in the presence and in the absence of USVC 6524. pA_2 values, defined as the negative logarithm of the molar concentration of antagonist which increases the dose ratio two-fold, were determined for USVC 6524. The numbers beside regression lines in Fig. 4 indicate slope and pA_2 value. The pA_2 values for USVC 6524 on the positive chronotropic and inotropic responses to isoprenaline were 7·69 and 7·52 respectively, with slopes of 0·97 and 1·15 respectively. The pA_2 value for USVC 6524 on the blood flow responses to isoprenaline was 8·62, with a slope of 0·92. All three regression lines have slopes very close to 1 and this supports the hypothesis of simple competitive antagonism between isoprenaline and USVC 6524.

The effects of USVC 6524 itself on resting cardiovascular parameters were determined in six dogs. Effects on mean arterial pressure, heart rate and myocardial contractility were determined 10 min after injection of each dose of USVC 6524, given at 15 min intervals. These results are summarized in Table 1. USVC 6524 produced a slight reduction in heart rate following a dose of 30 μg/kg, but there was no significant reduction in myocardial contractile force until after the injection of 1 mg/kg of USVC 6524. Following the injection of 100 μg/kg of USVC 6524, a dose that is approximately 10 times the amount needed to produce significant β-adrenoceptor blockade, mean arterial pressure was reduced to 89%, heart rate to 76%, and myocardial contractile force to 78% of their initial control values. The depressant effects of USVC 6524 on these cardiovascular parameters are relatively weak and occur only after the administration of relatively large β-adrenoceptor blocking doses of USVC 6524.

Effects of USVC 6524 in isolated tissues

The effects of USVC 6524 on isoprenaline inhibitory responses in the isolated rat uterus and guinea-pig trachea were determined. In both of these tissues USVC 6524 demonstrated a potent competitive β-adrenoceptor antagonism. Figure 6 demonstrates the β-adrenoceptor blocking action of USVC 6524 in three spontaneously contracting rat uterine segments. USVC 6524 in a concentration of 10^{-8}M abolished the inhibitory response to $1{\cdot}6 \times 10^{-5}$M papaverine. Any increase in the concentration of isoprenaline to 10^{-7}M produces an inhibitory response which is immediately abolished when 10^{-8}M USVC 6524 is subsequently added to the bath. The pA_2 values and slopes for USVC 6524 antagonism were determined in the quiescent rat uterus and the guinea-pig trachea and are shown in Table 2. The pA_2 values were obtained from regression lines calculated by the method of least

TABLE 1. *Cardiovascular effects of USVC 6524 in the anaesthetized dog*

Cardiovascular parameter	Control values*	After dose of USVC 6524 (μg/kg, i.v.) 3	10	30	100	300	1,000
Mean arterial pressure (mm Hg)	101·8 ±6·33	100·0 ±6·45	98·3 ±6·16	96·2 ±6·03	91·0† ±7·89	85·8† ±7·68	75·8† ±8·79
Heart rate (beats/min)	161·8 ±7·81	155·8 ±5·80	144·2 ±5·11	132·5† ±6·43	123·0† ±8·21	118·5† ±9·63	116·0† ±9·76
Contractile force (g)	158·5 ±12·87	152·7 ±14·84	142·5 ±14·78	135·2 ±15·61	124·8 ±17·26	121·0 ±18·22	121·2† ±7·93

* Means of six experiments ± S.E.M.
† $P<0{\cdot}05$. Responses after each dose of USVC 6524 were compared with the initial control value.

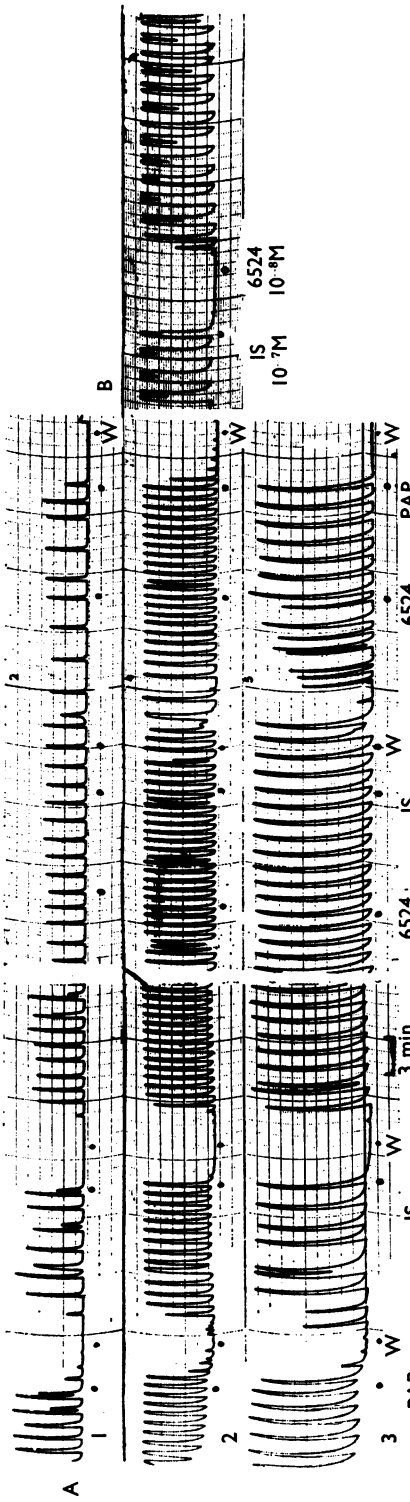

FIG. 6. Effects of USVC 6524 on the inhibitory responses induced by isoprenaline (IS) and papaverine (PAP) in the isolated, spontaneously contracting rat uterus. Panel A shows the effects of isoprenaline 10^{-8}M and of papaverine $1·6 \times 10^{-5}$M acting for 3 min before washing (W), before and after 10 min of pre-treatment with USVC 6524 (10^{-8}M) in three uterine segments. Panel B demonstrates the return of spontaneous motility by addition of USVC 6524 (10^{-8}M) after cessation of contractions induced by isoprenaline 10^{-7}M.

squares from pooled data obtained from eight preparations in each instance. A plot of log molar concentration of USVC 6524 versus log (concentration ratio −1) in which the concentration ratio was determined at the 50% points of the isoprenaline dose-response curves, yielded similar pA_2 values for USVC 6524 in the rat uterus and guinea-pig trachea of 9·04 and 9·32, respectively. The slopes of the regression lines were 0·80 in the rat uterus and 0·57 in the guinea-pig trachea.

Discussion

This study demonstrates that 1-isopropylamino-3-(4-indanoxy)-2-propanol HCl (USVC 6524) is a very potent β-adrenoceptor antagonist with relatively mild myocardial depressant effects. It is appropriate to compare USVC 6524 with propranolol. On the basis of dosage alone, USVC 6524 appears to be a more potent β-adrenoceptor antagonist than propranolol. Shanks (1966) described the β-adrenoceptor blocking actions of propranolol in the anaesthetized dog, utilizing a dose range of 0·1–1 mg/kg. USVC 6524 demonstrated a potent β-adrenoceptor blocking effect after doses of 3 and 10 μg/kg in our anaesthetized dogs. A more accurate comparison of β-adrenoceptor potency of USVC 6524 with that of propranolol may be obtained by comparing pA_x values. We have obtained pA_2 values of 7·69 and 7·52 for USVC 6524 on isoprenaline induced heart rate and myocardial contractile force responses with slopes approaching unity. Giudicelli, Schmitt & Boissier (1969) have determined pA_{10} values for propranolol on these parameters, in the anaesthetized dog, of 5·68 and 6·04 respectively, with slopes approaching unity. By extrapolation (addition of 0·95) pA_2 values for propranolol would thus theoretically be 6·63 and 6·95 respectively. This would suggest that USVC 6524 is approximately ten times more potent than propranolol as a β-adrenoceptor antagonist in the anaesthetized dog. The relative differences in pA_2 values for USVC 6524 on femoral flow, myocardial contractile force and heart rate, which are 8·62, 7·52 and 7·69 respectively, indicates a possible difference between cardiac and vascular β-adrenoceptors. Dose-response curves for isoprenaline on femoral flow were calculated from intra-arterial injections of isoprenaline. Vasodilator responses to isoprenaline, determined in this manner, reflected primarily a direct local effect on vascular β-adrenoceptors occurring before isoprenaline recirculated and produced systemic effects. In this way this technique approached more closely the situation that prevails when one uses isolated tissue systems for the determination of pA_2 values. The pA_2 values for the other cardiovascular parameters involved the intravenous injection of isoprenaline acting systemically rather than locally. Despite these factors the pA_2 values calculated represent a valid method of quantitating the β-adrenoceptor blocking properties of USVC 6524 in an *in vivo* situation. The possibility of fundamental differences between cardiac and vascular β-adrenoceptors suggested in this study is well supported by other studies which describe the ability

TABLE 2. *Slopes of regression lines and pA_2 values of USVC 6524 for isoprenaline antagonism in isolated tissues**

Tissue	pA_2 values	Slopes of regression lines
Rat uterus	9·04	0·80
Guinea-pig trachea	9·32	0·57

* Each value represents the pooled data obtained from eight tissue segments.

of practolol to block selectively cardiac but not vascular β-adrenoceptors and butoxamine to block selectively vascular but not cardiac β-adrenoceptors (Levy & Wilkenfeld, 1968).

The myocardial depressant properties of USVC 6524 are relatively mild, consisting of some bradycardia and hypotension ; but no significant negative inotropic effect occurs until after 1 mg/kg of USVC 6524, which is a dose 30–100 times its β-adrenoceptor blocking dose. Propranolol has been reported to produce a strong myocardial depression (Nakano & Kusahari, 1966 ; Blinks, 1967). Our results suggest that in the dog USVC 6524 is a more potent β-blocking agent with milder cardiovascular depressant properties than propranolol.

In the isolated rat uterus and guinea-pig trachea USVC 6524 demonstrated typical competitive β-adrenoceptor blocking actions of considerable potency. While the pA_2 values for USVC 6524 in the rat uterus and guinea-pig trachea were quite similar, 9·04 and 9·32 respectively, their regression line slopes of 0·80 and 0·57 were considerably less than 1. The implications of this finding are not clear. They probably do not reflect simple differences in diffusion of agonist and antagonist to the receptor. They do suggest the possibility of some differences between β-adrenoceptors which may be due simply to species differences.

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REFERENCES

AHLQUIST, R. P. (1948). A study of adrenotropic receptors. *Am. J. Physiol.*, **153**, 586–599.

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BLACK, J. W., CROWTHER, A. F., SHANKS, R. G. & DORNHORST, A. C. (1964). A new adrenergic β-receptor antagonist. *Lancet*, **2**, 1080–1081.

BLACK, J. W. & STEPHENSON, J. S. (1962). Pharmacology of a new adrenergic β-receptor blocking compound, Nethalide, *Lancet*, **2**, 311–314.

BLINKS, J. R. (1967). Evaluation of the cardiac effects of several β-adrenergic blocking agents. *Ann. N.Y. Acad. Sci.*, **139**, 673–685.

GIUDICELLI, J., SCHMITT, H. & BOISSIER, J. R. (1969). Studies on dl-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB 46) a new potent β-adrenergic blocking drug. *J. Pharmac. exp. Ther.*, **168**, 116–127.

LEVY, B. & WILKENFELD, B. E. (1968). An analysis of selective β-receptor blockade. *Eur. J. Pharmac.*, **5**, 227–234.

NAKANO, J. & KUSAHARI, T. (1966). Effect of β-adrenergic blockade on the cardiovascular dynamics. *Am. J. Physiol.*, **210**, 833–837.

PATTERSON, R. P. (1958). The tracheal strip: Observations on the response of tracheal muscle. *J. Allergy*, **29**, 165–172.

POWELL, C. E. & SLATER, I. H. (1958). Blockade of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. *J. Pharmac. exp. Ther.*, **122**, 480–488.

SHANKS, R. G. (1966). The effect of propranolol on the cardiovascular responses to isoprenaline, adrenaline and noradrenaline in the anaesthetized dog. *Br. J. Pharmac. Chemother.*, **26**, 322–333.

STANTON, H. C., KIRCHGESSNER, T. & PARMENTER, K. (1965). Cardiovascular pharmacology of two new β-adrenergic receptor antagonists. *J. Pharmac. exp. Ther.*, **149**, 174–182.

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