Br. J. Pharmac. Chemother. (1968), **33**, 552–559.

THE ACTIONS OF ORCIPRENALINE AND PROTOKYLOL ON GUINEA-PIG TRACHEA

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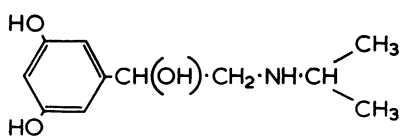
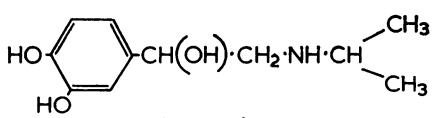
(*Received April* 9, 1968)

Orciprenaline and protokylol are two bronchodilators related in structure to isoprenaline and used clinically in the treatment of asthma. They have advantages over isoprenaline and adrenaline in their longer duration of action and greater stability which allows oral administration (Spitzbarth & Albers, 1961 ; Von Jacobi, Koch & Schleusing, 1963). The chemical structures of isoprenaline, orciprenaline and protokylol are illustrated in Fig. 1. Orciprenaline resembles isoprenaline except that the hydroxyl groups on the ring are in the 3 and 5 positions. Protokylol is a catecholamine with a larger group than isopropyl substituted on the nitrogen atom.

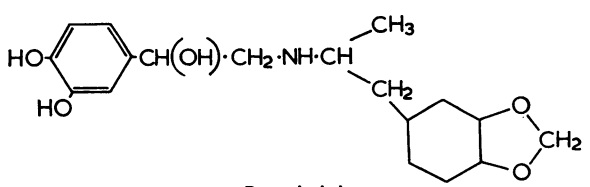
Orciprenaline and protokylol are reported to act purely on β-adrenoreceptors (Engelhardt, Hoefke & Wick, 1961 ; Von Jacobi *et al.*, 1963 ; Shanks, Brick, Hutchison & Roddie, 1967). Few quantitative studies of the effects of β-adrenoreceptor blocking



Orciprenaline



Isoprenaline



Protokylol

Fig. 1.

drugs on the action of these bronchodilators have been reported for isolated tissue. This paper describes a study of the potency of these two bronchodilators on the isolated guinea-pig tracheal chain preparation and the influence of the β-adrenoreceptor blocking drug, propranolol. To investigate the possible affinity of these drugs for uptake into adrenergic nerves in this tissue, the effect of cocaine on the actions of orciprenaline and protokylol has been studied.

METHODS

Relaxations of tracheal chain preparations from adult female guinea-pigs, weighing between 500 and 600 g, were recorded as described by Chahl & O'Donnell (1967). The preparations were suspended in Krebs bicarbonate solution containing ascorbic acid 200 μg/ml. Cumulative dose-response curves to orciprenaline and protokylol were obtained using the method of Van Rossum (1963). The effects of propranolol (10^{-3} mM) after contact for 1 hr with the trachea, and/or cocaine (10^{-2} mM) after contact for 0.5 hr, were tested on the responses to orciprenaline and protokylol. Cocaine and/or propranolol were replaced in the bath immediately after each washout.

Three series of experiments were carried out.

1. In nine experiments dose-response lines to orciprenaline and protokylol, added in varying order, were obtained before and after propranolol treatment.

2. In five experiments the same design was used except that cocaine was present in the bath fluid throughout the experiment.

3. Twenty-one experiments were designed to compare the activities of orciprenaline and protokylol with those of isoprenaline, adrenaline and noradrenaline in the presence of cocaine, before and after propranolol. Because a maximum of six dose-response lines could be obtained on any one preparation, only three of the five drugs could be tested before and after propranolol in any one experiment. The drugs used in each experiment were chosen and tested in varying orders and combinations.

To avoid drawing by eye or calculating individual dose-response lines and interpolating the ED50 (dose producing 50% maximum relaxation), we have used the following method. The paired doses and responses lying between 20 and 80% maximum, the straight portion of the line, for a particular drug and treatment, were pooled together. These were used in a linear least squares regression calculation where the regression of log dose on response was used. This enabled a value which we have called the mean log ED50 to be obtained and its standard deviation to be assessed from the scatter of all the points around the line. The method uses the actual experimentally determined points and gives an accurate representation of the mean log ED50 of the individual experiments. In most experiments the lines for any particular treatment group are almost parallel and not widely scattered. When this is so the slope of the calculated regression line gives a good representation of the slope of the individual lines. If individual lines are widely scattered, however, the slope of the calculated regression line may be altered. Thus comments on mechanisms of drug action which relate to slopes of dose-response lines have been made by reference to the slopes of individual experimental lines. The statistics calculated and the significance tests used have been described previously (Chahl & O'Donnell, 1967).

The results quoted for orciprenaline, protokylol, isoprenaline, adrenaline and noradrenaline in the presence of cocaine and cocaine plus propranolol were obtained from the combination of the series of five and twenty-one experiments. The series of nine experiments gave results for the normal orciprenaline and protokylol lines and those after propranolol treatment. Dose-response lines from some experiments were rejected because they did not have two points between 20 and 80% maximum. No normal lines, or lines in the presence of propranolol, for isoprenaline, adrenaline and noradrenaline were obtained in this study, so values quoted from Chahl & O'Donnell (1967) have been used.

Drugs

The following drugs were used: (−)-adrenaline acid tartrate, cocaine hydrochloride, (±)-isoprenaline sulphate, (−)-noradrenaline acid tartrate, (±)-orciprenaline sulphate (Alupent), (±)-propranolol hydrochloride, protokylol hydrochloride (Caytine, high melting point isomer, melting point, 170°–174° C).

RESULTS

Dose-response lines for orciprenaline and protokylol

Typical dose-response lines to orciprenaline, protokylol and isoprenaline are shown in Fig. 2. The relaxation produced by each dose of orciprenaline developed more slowly than that produced by each dose of isoprenaline. The effect of protokylol was often slightly slower in developing than that of isoprenaline. The maximum response of the preparation to orciprenaline and protokylol was the same as that to isoprenaline, adrenaline and noradrenaline. Orciprenaline and protokylol produced mean log dose-response lines which were not significantly different in slope from those produced by isoprenaline, adrenaline or noradrenaline. This reflected the observations in individual experiments except that, as can be seen in Fig. 2, there was a tendency on some preparations for the orciprenaline lines to be slightly less steep.

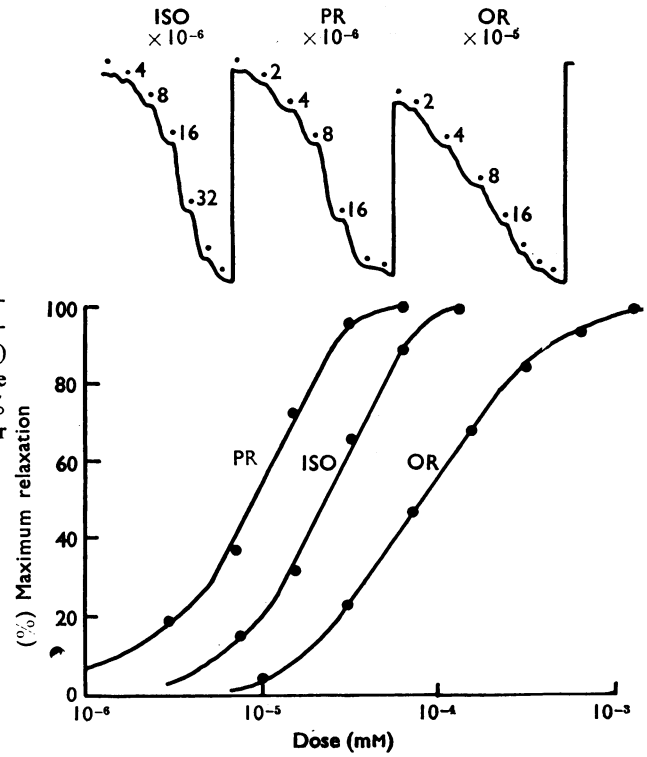


Fig. 2. Upper trace shows cumulative dose-response lines to isoprenaline (ISO), protokylol (PR) and orciprenaline (OR). These lines are plotted as log dose-% maximum relaxation in the lower graph.

Because the maximum responses and the slopes of the calculated log dose-response lines were the same for orciprenaline, protokylol, adrenaline, noradrenaline and isoprenaline, the mean log ED50s have been used to compare potencies. The normal mean log ED50s and their standard deviations for the five drugs are shown in Table 1. Protokylol was the most potent drug of the series, being significantly more potent than isoprenaline ($t=3.15$; $P=0.01$–0.001). Orciprenaline was slightly less potent than adrenaline ($t=2.13$; $P=0.05$–0.02). Noradrenaline was least potent. The ratio of potencies of these five drugs was protokylol : isoprenaline : adrenaline : orciprenaline : noradrenaline = 159.2 : 74.7 : 7.7 : 4.1 : 1.

TABLE 1

MEAN LOG ED50 OF ORCIPRENALINE, PROTOKYLOL, ISOPRENALINE, ADRENALINE AND NORADRENALINE

S.D., standard deviation. Number of experimental lines are in parentheses. * Values quoted from Chahl & O'Donnell (1967).

Drug		Mean log ED50 ± S.D.
Protokylol (PR)		−5·45±0·16 (8)
Isoprenaline (ISO)		−5·12±0·26 (16)*
Adrenaline (A)		−4·14±0·32 (17)*
Orciprenaline (OR)		−3·86±0·28 (9)
Noradrenaline (NA)		−3·25±0·22 (15)*
Comparisons	*t* Values	*P*
PR and OR	13·28	<0·001
PR and ISO	3·15	0·01–0·001
A and OR	2·13	0·05–0·02

Effect of cocaine and propranolol

The mean log ED50s of orciprenaline and protokylol in the presence of cocaine (10^{-2} mM), propranolol (10^{-5} mM) and cocaine plus propranolol are shown in Table 2. Cocaine produced no potentiation of orciprenaline or protokylol. Propranolol caused a significant block of orciprenaline and protokylol and the mean log ED50s of the propranolol treatment lines were insignificantly different from those obtained with cocaine also present.

TABLE 2

EFFECT OF PROPRANOLOL AND/OR COCAINE ON THE POTENCIES OF ORCIPRENALINE AND PROTOKYLOL

S.D., standard deviation. Number of experimental lines are in parentheses. * Highly significant difference ($P<0·001$).

	Mean log ED50 ± S.D.	
Treatment	Orciprenaline	Protokylol
Control (N)	−3·86±0·28 (9)	−5·45±0·16 (8)
Propranolol (P)	−1·39±0·35 (7)	−3·11±0·39 (6)
Cocaine (C)	−3·78±0·24 (15)	−5·37±0·30 (15)
Propranolol+cocaine (P+C)	−1·29±0·22 (16)	−2·90±0·27 (15)
Comparisons (*t* values)		
N and P	14·67*	14·20*
N and C	0·71	0·67
P and P+C	0·79	1·34

Degree of block by propranolol (10^{-5} mM)

The degree of block of the effects of orciprenaline, protokylol, adrenaline, noradrenaline and isoprenaline produced by propranolol in the presence and absence of cocaine in the bath fluid are shown in Table 3. The degree of block in the absence of cocaine was measured as the difference between the mean log ED50s of the normal and propranolol treatment lines. The degree of block when cocaine was present was measured as the difference between the mean log ED50s of the cocaine and the cocaine plus propranolol treatment lines.

In the absence of cocaine, the effects of adrenaline, orciprenaline and protokylol were blocked by propranolol to a similar extent. The effect of noradrenaline was blocked least. In the presence of cocaine, the effects of orciprenaline, protokylol, isoprenaline

TABLE 3

DEGREE OF BLOCK BY PROPRANOLOL (10^{-3} mM)

Expressed as the difference between the mean log ED50 with and without propranolol treatment. S.D., Standard deviation of the difference. The numbers of experimental lines contributing to each mean log ED50 are shown in parentheses. * Values quoted from Chahl & O'Donnell (1967). † Highly significant difference ($P<0.001$).

Drug	Degree of block (log units) ± S.D. Normal	Cocaine (10^{-3} mM)
Adrenaline (A)	2·53±0·28* (17, 12)	2·84±0·27 (15, 15)
Orciprenaline (OR)	2·47±0·33 (9, 7)	2·49±0·24 (16, 16)
Protokylol (PR)	2·34±0·31 (8, 6)	2·47±0·30 (15, 15)
Isoprenaline (ISO)	2·16±0·26* (16, 8)	2·46±0·25 (11, 11)
Noradrenaline (NA)	1·68±0·25* (15, 5)	2·46±0·25 (12, 12)
Comparisons (*t* values)		
A and OR	0·62	5·22†
OR and PR	1·03	0·28
PR and ISO	1·84	0·12
ISO and NA	5·95†	0·00

and noradrenaline were blocked by propranolol to the same extent. The effect of adrenaline was blocked more. This was observed in individual experiments even though there was some variation in the degree of block of the effects of a particular drug depending on its order of addition after propranolol. Cocaine did not alter the degree of block of the effects of orciprenaline ($t=0.23$) or of protokylol ($t=1.28$) produced by propranolol, but it increased the degree of block of the effects of adrenaline, noradrenaline and isoprenaline.

DISCUSSION

Orciprenaline was found to be slightly less potent as a tracheal relaxant than was adrenaline. Its bronchodilator effect *in vivo* has been reported to be similar to that of adrenaline (Engelhardt *et al.*, 1961) and weaker than that of isoprenaline (Shanks *et al.*, 1967). The finding that protokylol is twice as potent as isoprenaline does not agree with Chen (1956) or Ariens (1967). Chen found protokylol and isoprenaline to be equipotent on guinea-pig trachea, whereas Ariens, using stimulated calf tracheal muscle, reported that the high melting point isomer of protokylol was eight times more potent than isoprenaline, whereas the low melting point isomer was equipotent. The powerful activity of protokylol on the trachea would add support to the reports that it has a better bronchodilator effect in guinea-pig asthma induced by histamine than has isoprenaline (Von Jacobi *et al.*, 1963). Clinical studies describe protokylol as being equieffective with isoprenaline (Leslie & Simmons, 1957). On the rabbit atria, protokylol was found to be less potent than isoprenaline in increasing the rate (Giles & Miller, 1967). This suggests that protokylol, used as a bronchodilator, might have a more favourable ratio of effects on β-receptors of bronchi and heart than has isoprenaline.

The intrinsic activities of orciprenaline, protokylol, isoprenaline, adrenaline and noradrenaline have been assumed to be the same, for it is not known whether a receptor reserve exists in the trachea. Knowledge of the percentage of the receptors involved in

producing a maximum response of the tissue to β-adrenoreceptor agonists would be difficult to obtain at the present time because there is no known noncompetitive β-receptor blocking drug. When a receptor reserve is present, drugs producing the same maximum response might have different intrinsic activities (Ariens, Simonis & Van Rossum, 1964). Stephenson (1956) has shown theoretically that, for drugs producing the same maximum response but having different efficacies and the same affinity, the apparent affinity (as measured by the ED50) decreases as the efficacy decreases. Therefore, when the true efficacies and affinities of a series of drugs, all producing the same maximum response, is unknown, it is difficult to decide conclusively whether a particular structure is important for determining affinity or efficacy. Nevertheless, orciprenaline and protokylol are both potent relaxants of the guinea-pig trachea, which confirms that a large substitution on the nitrogen atom is advantageous for β-receptor activity. Because orciprenaline is less potent than isoprenaline, it seems that the 3 and 4 positions for hydroxyl groups on the benzene ring are more favourable than the 3 and 5 positions. The fact that protokylol is slightly more potent than isoprenaline suggests that increasing the size of the substitution on the nitrogen atom beyond isopropyl leads to an increase in activity.

Neither orciprenaline nor protokylol was potentiated by cocaine. The suggests that these drugs are not taken up by adrenergic nerve terminals in guinea-pig trachea. Foster (1967) reported that cocaine did not potentiate isoprenaline on this preparation. Further experiments carried out in this laboratory (Chahl & O'Donnell, unpublished results) indicate that the small potentiation of isoprenaline observed in previous experiments (Chahl & O'Donnell, 1967) might not be significantly different from the slight changes in tissue sensitivity which can occur during long-term experiments. Thus increase in the size of the substitution on the nitrogen atom appears to decrease the affinity of drugs for the cocaine-sensitive uptake mechanism in guinea-pig trachea. The structure-activity characteristics of the uptake mechanism in guinea-pig trachea might be similar to those described by Iversen (1965) for Uptake 1 in the rat heart.

In individual experiments, propranolol caused a parallel shift in the log dose-response lines to both orciprenaline and protokylol. Thus it can be assumed that these drugs act on β-adrenoreceptors. The degree of block by propranolol of the effects of the five drugs studied was not the same unless cocaine was also present in the bath fluid. We assume that cocaine blocks the uptake into adrenergic nerve terminals of those drugs which have an affinity for this mechanism, thus allowing drugs to compete equally with propranolol for the β-receptors. A tendency for the effects of adrenaline to be blocked to a rather greater extent than those of the other drugs under these conditions, however, was again observed in these experiments. Chahl & O'Donnell (1967) suggested that a second uptake or "site of loss" might operate in the trachea when the cocaine-sensitive uptake was saturated. Adrenaline would need to have a higher affinity for this site. It is possible that the greater block of adrenaline could be the result of a non-specific adherence of adrenaline to tissue, possibly the mucopolysaccharides of the tracheal cartilage. A more rapid loss of activity of adrenaline by auto-oxidation in the bath seems unlikely. Ascorbic acid was present in the Krebs fluid during experiments, but this could be insufficient to prevent auto-oxidation. Assay of the contents of the bath fluid for 40 min at 5 min intervals after the addition of adrenaline, however, showed insignificant loss of activity. Haggendal & Johnsson (1967) have shown that the presence of a piece of tissue (in their experiments,

rat diaphragm) prevented destruction of noradrenaline in a Krebs bicarbonate buffer (pH 7.4) gassed with 96% oxygen and 4% carbon dioxide at 37° C.

There is also the possibility that some α-receptors, activation of which causes contraction, may be present in the guinea-pig trachea. Foster (1967) could not detect α-receptors in this preparation, but we have previously observed that a contraction of the tissue can occur at the beginning of a dose-response line to adrenaline or noradrenaline in the presence of propranolol—that is, when high doses of catecholamines are used (Chahl & O'Donnell, 1967). Takagi, Osada, Takayanagi & Taga (1967) have also recently shown the existence of α-receptors in the guinea-pig trachea. Thus the α-receptors might be activated by high doses of catecholamines such as are used in the presence of a β-receptor blocking drug and thus antagonize the relaxation produced by activation of β-receptors. On guinea-pig trachea, the excitatory potency on α-receptors, found in the presence of a β-receptor blocker, was adrenaline>noradrenaline>phenylephrine (Takagi *et al.*, 1967). Thus, if adrenaline were a more potent α-receptor stimulant it would be possible that, in the presence of the dose of propranolol we used, slightly more adrenaline might be needed to obtain a dose-response line. This could result in a greater block of the effects of adrenaline than of the other drugs.

SUMMARY

1. Relaxations of the isolated guinea-pig tracheal chain produced by orciprenaline and protokylol developed more slowly than that produced by isoprenaline, adrenaline or noradrenaline, but all produced the same maximum response.

2. Protokylol (high melting point isomer) was slightly more potent than (±)-isoprenaline, and (±)-orciprenaline was slightly less potent than (−)-adrenaline.

3. Orciprenaline and protokylol were not potentiated by cocaine (10^{-2} mM) and are considered to have no affinity for uptake into adrenergic nerves in the guinea-pig trachea.

4. Orciprenaline and protokylol were blocked by propranolol (10^{-3} mM) to the same extent as were isoprenaline and noradrenaline, if cocaine was in the bath. They were blocked to a smaller extent than was adrenaline and the significance of this is discussed.

Support for this work by grants from the Asthma Foundation of Queensland and the National Health and Medical Research Council of Australia is gratefully acknowledged. We would like to thank Miss Sandra L. Whyte for technical help and the following for gifts of drugs: Dr. H. L. Friedman, Lakeside Laboratories, Milwaukee, for protokylol; Dr. W. Graubner and Dr. K. Higgins, Boehringer Ingelheim, for orciprenaline; and Imperial Chemical Industries for propranolol. This work was presented in part at the first meeting of the Australasian Society of Clinical and Experimental Pharmacologists in Melbourne, Australia, November 29–30, 1967.

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