New selective β_2-adrenoceptor stimulants 5-(1-hydroxy-2-isopropylaminobutyl)- and 5-(1-hydroxy-2-ethylaminobutyl)-8-hydroxycarbostyril hydrochlorides (OPC-2009 and OPC-2030) and cyclic AMP concentration

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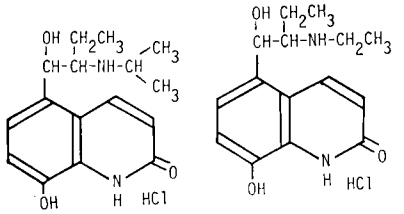
Catecholamines are widely used for the treatment of reversible airway obstruction. Isoprenaline is effective when given by aerosol or sublingual routes. It can, however, cause marked side effects due to stimulation of adrenoceptors of the β-type in the cardiovascular system. Of the many derivatives of carbostyril synthesized by Yoshizaki, Tanimura & others (1976), OPC-2009 and OPC-2030 were chosen, because they seem to have a more selective action on tracheal smooth muscle than on cardiac muscle. In this paper we report studies of their modes of action on the tracheal muscle of guinea-pig.



OPC-2009 OPC-2030

OPC-2009 (5-(1-Hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride). OPC-2030 (5-(1-Hydroxy-2-ethylaminobutyl)-8-hydroxycarbostyril hydrochloride).

Isolated tracheal preparations and atria were suspended in a 30 ml organ bath filled with Locke Ringer solution kept at 37 ± 0·5° and gassed with a mixture of 5% CO_2 in oxygen.

Tracheal preparation. Tracheae were removed from male guinea-pigs, 300 to 400 g. The isolated trachea was helically cut. The test drugs were applied to the tracheal preparation contracted by histamine (3×10^{-5} M). Inhibitory responses to the test drugs were recorded isotonically and expressed as percentage relative to the maximum relaxation produced by isoprenaline.

Atria. Atria were also removed from male guinea-pigs, 300 to 400 g. Isometric responses to the test drugs were observed and expressed as percentage relative to the maximum increase of tension induced by isoprenaline (Takagi & Takayanagi, 1970). Initial tension was 1·0 g.

All drugs were applied cumulatively. The activity of each drug was expressed as a pD_2-value which is the negative logarithm of the molar concentration which produces half the maximum response (van Rossum, 1963). Antagonisms between β-adrenoceptor stimulants (the test drugs) and a β-adrenoceptor blocker, propranolol, were tested on tracheal preparations. After control concentration action curves of the β-stimulants had been obtained, the concentration action curves of the β-stimulants in the presence of propranolol (3×10^{-7} M 5 min pretreatment) were again obtained on the tracheal preparation. Competitive antagonistic activity of propranolol against each drug is expressed as a pA_2-value (Schild, 1947). The pA_2 value was calculated from the parallel shift of the concentration action curves of the drugs according to van Rossum (1963).

The Locke Ringer solution used contained (mM) NaCl 147, KCl 5·1, $CaCl_2$ 2·1, $MgCl_2$ 2·1, $NaHCO_3$ 9·6 and glucose 2·8. All the results obtained on the isolated organs presented are means of at least 8 experiments.

The sample for estimation of cyclic (c)AMP was prepared by the methods of Ohkubo, Takayanagi & Takagi (1976). The trachea was isolated from guinea-pigs, 250 to 300 g. The smooth muscle was cut carefully from connective tissues and chondrins. Only smooth muscle was used as a test preparation. Since the variance in the cAMP concentration between batches of guinea-pigs is larger than that within a batch, the smooth muscle isolated from one animal was divided into two: one half was used for measuring the control concentration of cAMP and the other for estimating any change of cAMP concentration after drug treatment. After experimental treatment the smooth muscle was placed in liquid nitrogen and homogenized with a glass homogenizer in 2 ml of cold 6% trichloroacetic acid. The homogenate was centrifuged at 1000 *g* at 0° for 30 min and the supernatant was acidified with 1 N HCl, thereafter the trichloroacetic acid layer was extracted 4 times with 3 volumes of ether. The cAMP sample was lyophylized. The lyophylized samples were dissolved with distilled water and these were applied to Sephadex G-25 (0·9 × 2·4 cm) columns, which were equilibrated with 1·2 ml water and cAMP was eluted with 2·5 ml water. cAMP was lyophylized to dryness and redissolved with 200 μl of 50 mM sodium acetate buffer (pH 6·2) and assayed according to Gilman (1970).

To estimate cAMP phosphodiesterase activity, the tracheal smooth muscle, which was dissected free from connective tissues and chondrins as described, was homogenized in 8 volumes of 25 mM tris-HCl with

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Polytron with the rheostat setting on 8 for 5 s three times. The homogenate was centrifuged at 1000 *g* for 15 min at 0 to 4°. The 1000 *g* supernatant was used as a crude enzyme preparation. cAMP phosphodiesterase activity was estimated with radioactively labelled cAMP (adenosine 8-labelled ^{3}H-3′,5′-cyclic monophosphate) as a substrate. An appropriately diluted enzyme preparation was incubated in 0·4 ml of 40 mM tris-HCl buffer (pH 7·5) containing 5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 10^{-7} M ^{3}H-cAMP and 10^{-6} and 10^{-5} M cAMP. After 10 min incubation at 30° the reaction was terminated by boiling for 3 min. Then 5′-nucleotidase (0·1 unit ml^{-1}) was added to the reaction mixture at 37° for 10 min to change 5′-AMP into adenosine. The reaction products were separated by paper chromatography (solvent: a mixture of ethanol 70 ml and 1 M ammonium acetate, 30 ml) and the radioactivity of unhydrolysed cAMP was counted in 10 ml of scincillation fluid. The ratio of hydrolysis of cAMP was adjusted from 20 to 60% in control samples (Inatomi, Takayanagi & Takagi, 1975).

The amount of protein contained in the incubation fluid was determined according to Lowry, Rosebrough & others (1951), using bovine serum albumin as a standard.

All the test drugs relaxed the tracheal preparation contracted by histamine (3×10^{-5} M); the pD_2-values (mean ±s.e.) were 8·1 ± 0·2 for OPC-2009, 7·7 ± 0·2 for OPC-2030, 6·9 ± 0·1 for salbutamol and 7·6 ± 0·2 for isoprenaline. The maximum responses to OPC-2009, OPC-2030 and salbutamol were the same as that to isoprenaline. Of the 4 drugs, OPC-2009 was the most potent. On the other hand the pA_2-values (mean ± s.e.) of propranol were 7·6 ± 0·2 against isoprenaline, 7·7 ± 0·1 against OPC-2009, 7·8 ± 0·1 against OPC-2030 and 7·7 ± 0·1 against salbutamol, suggesting that the site of action of OPC-2009 and OPC-2030 is the same as that of isoprenaline and salbutamol, which is the β-adrenoceptors.

All the drugs tested increased the spontaneous contractile force of the isolated atria; the pD_2-values were 7·2 ± 0·3 for OPC-2009, 7·2 ± 0·3 for OPC-2030, 6·1 ± 0·2 for salbutamol and 8·5 ± 0·1 for isoprenaline. The concentration action curves for OPC-2009, OPC-2030 and salbutamol were less steep than that for isoprenaline and the maximum responses (mean ± s.e.) relative to that (100%) to isoprenaline were 39 ± 5% for OPC-2009, 21 ± 8% for OPC-2030 and 58 ± 11% for salbutamol. Of the 4 drugs used, isoprenaline was the most potent on the atria.

Time response relations of the actions of OPC-2009 and OPC-2030 on isotonic relaxation of the trachea and cAMP content of the tracheal smooth muscle were examined. Relaxation induced by OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M) was observed only to the extent of about 6% of the maximum amplitude at 30 s after application of OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M). The tracheal preparation relaxed

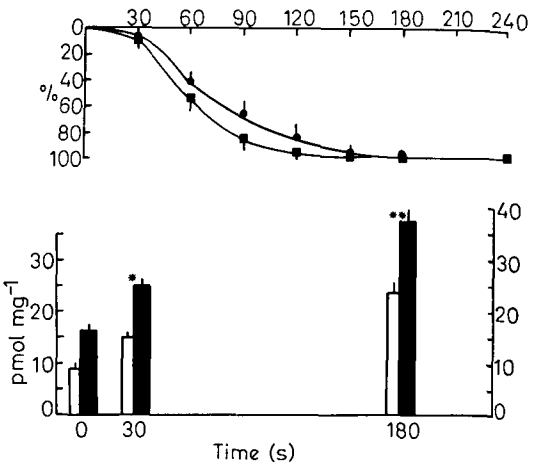

FIG. 1. Time response relation of the actions of OPC-2009 and OPC-2030 on isotonic relaxation (%) of a tracheal preparation (upper) and cAMP concentration of (pmol mg^{-1} protein) tracheal smooth muscles (lower). OPC-2009 and OPC-2030 were added to the medium at zero time. *P*-values were determined by comparing the cAMP concentrations in the tracheal smooth muscle treated with OPC-2009 or OPC-2030 with the corresponding control concentrations (column at zero time) $*P < 0.05$, $** P < 0.01$. Each value is presented as mean ± s.e. Open column: OPC-2009 (10^{-6} M), closed column: OPC-2030 (3×10^{-6} M). ●—OPC-2009 (10^{-6} M), ■—OPC-2030 (3×10^{-6} M).

to 90% or more of the maximum amplitude at 180 s after application of OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M). Several minutes were needed for the tracheal preparation to relax to the maximum amplitude (Fig. 2). In separate experiments we estimated the cAMP contents in the tracheal smooth muscle at 30 s and 180 s after application of 10^{-6} M of OPC-2009 or 3×10^{-6} M of OPC-2030. The 30 s and 180 s incubation of the tracheal smooth muscle with OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M) significantly increased the tissue content of cAMP (Fig. 1).

The effect of propranolol on the increase of cAMP induced by OPC-2009 or OPC-2030 was tested. Tracheal smooth muscle was incubated for 3 min after application of OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M), and propranolol (10^{-6} M) was applied 5 min before the application of OPC-2009 or OPC-2030. The experimental results are summarized in Table 1. The increases of the tissue cAMP contents produced by OPC-2009 (10^{-6} M) and OPC-2030 (3×10^{-6} M) were completely blocked by a β-adrenoceptor blocking drug, propranolol, which was without any effect on the basal content of cAMP, indicating that the increases of cAMP content by OPC-2009 and OPC-2030 were mediated by the β-adrenoceptors.

When cAMP at 10^{-6} and 10^{-5} M was used as substrates, OPC-2009 in 10^{-6} M and OPC-2030 in 3×10^{-6} M, which are concentrations that produce the maximum

Table 1. *Effect of propranolol on the increase of cAMP induced by OPC-2009 or OPC-2030 in tracheal smooth muscle.*

Treatment	cAMP content mean ± s.e.	No of exps	*P* values
Control (untreated)	100	25	
OPC-2009 (10^{-6} M)	181 ± 13	5	<0.01
OPC-2030 (3×10^{-6} M)	197 ± 21	5	<0.01
Propranolol (10^{-6} M)	112 ± 16	5	
OPC-2009 (10^{-6} M) + propranolol (10^{-6} M)	106 ± 18	5	
OPC-2030 (3×10^{-6} M) + propranolol (10^{-6}M)	96 ● 15	5	

cAMP content is expressed as a percentage of cAMP content (20·6 ± 2·1 pmol mg^{-1} protein) in the untreated tracheal muscle.

relaxation of the trachea, were without any effect on cyclic AMP-phosphodiesterase activity. Papaverine in 10^{-5} M produced 75% or more inhibition of cAMP phosphodiesterase activity of the tracheal preparation as reported by Inamasu, Shinjo & others (1974).

The pharmacological results presented show OPC-2009 and OPC-2030 to be β-adrenoceptor stimulants. It is clear, however, that OPC-2009 and OPC-2030 are more active on tracheal smooth muscle than on atria. These results indicate that OPC-2009 and OPC-2030 are selective β_2-receptor stimulants according to the proposal of Lands, Arnold & others (1967) who subdivided β-adrenoceptors into β_1-receptors in cardiac muscle and β_2-receptors in bronchial muscle.

The present results concerned with salbutamol and isoprenaline are similar to those of Cullum, Farmer & others (1969) and Raper & Malta (1973). In the guinea-pig isolated atria, salbutamol, OPC-2009 and OPC-2030 were partial agonists in the present study. The maximum responses of the atria to OPC-2009 and OPC-2030 are smaller than that to salbutamol, while the effective concentrations of salbutamol were larger than those of OPC-2009 and OPC-2030. It is, therefore, difficult to give potency ratios for OPC-2009, OPC-2030 and salbutamol in the isolated atria.

The increases of cAMP levels produced by OPC-2009 and OPC-2030 were inhibited by propranolol. Furthermore, OPC-2009 and OPC-2030 did not affect the cAMP phosphodiesterase activity. The increases of cAMP concentration induced by OPC-2009 and OPC-2030 are likely to be a result of activation of tissue adenyl cyclase. Notwithstanding that relaxation of the tracheal preparation at 30 s after addition of OPC-2009 (10^{-6} M) or OPC-2030 (3×10^{-6} M) was observed only to the extent of about 6% of the maximal magnitude, which was not significantly different from the base line, tissue concentrations of cAMP were significantly increased by the 30 s incubations. The significant increase of cAMP during the short term incubation in the presence of the β-adrenoceptor stimulants is consistent with the results obtained on guinea-pig trachea (Inamasu & others, 1974), guinea-pig taenia caecum (Bueding, Butcher & others, 1966), rabbit colon (Andersson, 1972) and rat uterus (Marshall & Kroeger, 1973), and suggests that the action of OPC-2009 and OPC-2030 are mediated by the increase in the cAMP concentration.

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