STUDIES ON TETRAHYDROISOQUINOLINES (THI) (I) BRONCHODILATOR ACTIVITY AND STRUCTURE- ACTIVITY RELATIONSHIP

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For many years the synthesis of isoquinoline derivatives has been carried out in our Organic Chemistry Research Laboratory. In the process of pharmacological screening tests, 1-(3,4-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was found to have significant bronchodilating as well as hypotensive actions, as briefly communicated in the previous report (1).

This compound had been synthesized by Pyman in 1909 (2) and its hypotensive action was reported by Laidlaw in the following year (3). While our work was in progress, Holtz and his co-workers (4) demonstrated the formation of this compound as a condensation product of dopamine and its enzymatically deaminated product, 3,4-dihydroxyphenylacetaldehyde. They also reported its hypotensive and bronchodilating activities, which were interpreted as a β -sympathomimetic action of this compound.

In reference to these studies 1-alkyl derivatives of tetrahydroisoquinoline (THI) were synthesized and tested their bronchodilating activities. Among the compounds tested, 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (IX) was observed to be the most active bronchodilator hitherto described in the literature.

The relationship between the chemical structure and the bronchodilating activity was investigated in this paper.

MATERIALS AND METHODS

Isolated tracheal muscle preparations of guinea-pig were used to estimate the bronchodilator activity. Guinea-pigs of 300 to 400 g body weight were killed by stunning and bleeding from the femoral artery. The whole length of the trachea was removed, placed in Tyrode's solution at room temperature, and carefully cleaned of connective tissue. The trachea was sectioned into 12 to 14 rings of equal width and each ring was opened by cutting through the cartilage opposite to the muscular portion. A so called "tracheal chain" [Takagi *et al.* (5)] was made by tying 6 to 7 pieces end to end so the muscular portion of each piece was aligned. The chains were placed in fresh Tyrode's solution and left over night at about 5°C in an ice box. For the test one of the chains was suspended in an organ bath filled with Tyrode's solution at $37 \pm 1^\circ\text{C}$, into which air was continually bubbled. The responses of the chain were recorded on a kymograph by means of an isotonic lever.

The bronchodilator activity was assessed by drawing a cumulative dose-response curve of the compound tested, and expressed in terms of the pD_2 value (6,7). The pD_2 value was the negative logarithm of the molar concentration that caused 50% relaxation of the tracheal chain present in a state of maximal contraction provoked by histamine dihydrochloride at a final concentration of 1×10^{-5} g/ml.

The values in the tables represent the means of pD_2 values obtained in at least eight, but as a rule more than ten, experiments.

The drugs used in the present work were as follows; epinephrine hydrochloride (E) (Sankyo), isoproterenol sulfate (Iso) (Boehringer Sohn), histamine dihydrochloride (Hist) (Takara) and pronethalol (I.C.I.). The isoquinoline derivatives were supplied by our Organic Chemistry Research Laboratory.

RESULTS

1. Bronchodilator activities of 1-substituted-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines

As shown in Table I, four classes of substituents were introduced at position 1 of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, namely 1) aryl, 2) arylmethyl, 3) 2-arylethyl, and 4) 3-arylpropyl. Although the bronchodilator activities of 1-alkyl derivatives of tetrahydroisoquinoline have already been reported briefly by Craig *et al.*, (8), the isopropyl

TABLE I. Bronchodilator activity of 1-substituted-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines.

Compound No.	R	pD_2	$\pm$ S.E.	Potency ratio
I		<3	—	<0.01
II		<3	—	<0.01
III		<3	—	<0.01
IV		<3	—	<0.01
V		<3	—	<0.01
VI		<3	—	<0.01
VII		6.12	0.07	17
VIII		6.45	0.06	37

IX		8.81	0.12	8,500
X		7.19	0.12	200
XI		6.53	0.19	45
XII		<3	—	<0.01
XIII		<3	—	<0.01
XIV		<3	—	<0.01
XV		<3	—	<0.01
XVI		6.86	0.09	96
XVII		3.80	0.07	0.08
	Epinephrine	6.88	0.04	100
	Isoproterenol	7.88	0.05	1,000

Isolated tracheal chains of guinea-pig were used to estimate the bronchodilator activity (*See Methods*). The pD_2 -value was the negative logarithm of the molar concentration that caused 50% relaxation of the tracheal chain. The values represent the mean of pD_2 -values obtained in at least eight, but as a rule more than ten, experiments.

analogue, reported as the most active compound among them and the *n*-propyl analogue, reported as slightly active, were also added in the table for the comparison. Among the compounds tested, the isopropyl analogue (XVI) was as active as E, while arylmethyl analogues (VII-XI) were 0.2 to 85 times as active as E. It is worth noting that the 3,4,5-trimethoxybenzyl analogue (IX) was 85 times as active as E and about ten times as active as Iso. However, other compounds, i.e., aryl, 2-arylethyl, and 3-arylpropyl derivatives, were all far less active, one ten thousandth of E, and *n*-propyl analogue (XVII) was practically inactive.

The 4-pyridylmethyl analogue (X), one of the arylmethyl derivatives, was highly active. However, saturation as the pyridyl moiety resulted in an enormous reduction of activity, so that the 4-piperidylmethyl analogue (XII) was inactive, as was also the case with the relationship between the activity of 3-pyridylmethyl (XI) and of 3-piperidylmethyl (XIII) analogues. In other words, by replacement of the aromatic ring with the aliphatic ring the activity was abolished.

The results shown in Table 1 indicate that the presence of an aromatic ring, attached to the tetrahydroisoquinoline through one methylene group at position 1, is essential for bronchodilator activity.

2. *Effects of substitution at position 6 and/or 7 on bronchodilator activity*

It is tentatively accepted that the catechol group of E and Iso is essential for the β -sympathomimetic actions, e.g., bronchodilator activity [Ariens (11), Belleau (12), Pratesi *et al.* (13)]. If the chemical structures of our compounds (Table 1, Fig. 3) are compared with those of E and Iso, it may be seen that they have the catechol group in the analogous position. Consequently the role of this catechol group in the bronchodilator activity was investigated with some derivatives of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (VII) which was moderately active. Since this compound could be considered chemically as the prototype of the bronchodilating agents of isoquinoline series, the compounds listed in Table 2 were tested. In a series of 6-and/or 7-substituted compounds, only the 6,7-

TABLE 2. Effect of hydroxyl group(s) at position 6 and/or 7 on bronchodilator activity.

Compound No.			pD ₂	± S.E.	Potency ratio Epi. = 100
	R ₁	R ₂			
XVIII	OH	H	3.28	0.09	0.03
XIX	H	OH	3.23	0.06	0.02
VII	OH	OH	6.12	0.07	17
XX	OCH ₃	OCH ₃	3.10	0.05	0.02
XXI	-O-CH ₂ -CH ₂ -O-		<3	—	<0.01
	Epinephrine		6.88	0.04	100

dihydroxy analogue was found to be active and loss of the free catechol moiety, for instance, by replacement of either of the two hydroxyl groups with hydrogen (XVIII, XIX), or by masking both hydroxyl groups with O-methyl (XX) or ethylene group (XXI), led to an activity loss to a great extent. No difference has been found between the contribution of the 6- and 7-hydroxyl groups to show the activity.

It can be concluded therefore that in these compounds integrity of the catechol portion is a requirement for manifestation of the activity, as has been well known in the case of catecholamines.

3. *Bronchodilator activity and structures of B-ring of isoquinoline*

Since the B-ring of THI was assumed to be one of the active sites, the relationship between substitution of hydrogen atoms of this ring and change in activity was investigated. It can be seen from Table 3 that a remarkable loss of activity resulted from such changes as introduction of 1) a double bond between position 1 and 2 (XXII), 2) a methyl group at position 2 (XXIII), or 3) a methyl group at position 3 (XXIV).

These results suggest that the 1,2,3,4-tetrahydroisoquinoline structure is of great importance to the activity.

TABLE 3. Bronchodilator activity and structures of B-ring of isoquinoline.

Compound No.			pD ₂	± S.E.	Potency ratio Epi. = 100
	R ₁	R ₂			
VII	H	H	6.12	0.07	17
XXII	<i>Δ</i> ^{1,2}	H	3.22	0.07	0.02
XXIII	CH ₃	H	3.17	0.05	0.02
XXIV	H	CH ₃	3.27	0.05	0.03
XXV	<i>Δ</i> ^{1,2}	CH ₃	4.01	0.11	0.13
Epinephrine			6.88	0.04	100

TABLE 4. Optical isomers of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and bronchodilator activity.

Optical rotation	pD ₂	± S.E.	Potency ratio	
racem	6.12	0.07	17	100
<i>l</i> -	6.52	0.15	44	250
<i>d</i> -	3.12	0.09	0.02	0.1
Epinephrine	6.88	0.04	100	580

4. Optical isomers of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and bronchodilator activity

It is generally known that one of the optical isomers of a pharmacological agent is not only more active than the antipode but also sometimes antagonized by it. Since 1-arylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines have one asymmetric carbon at position 1, differences in β -sympathomimetic potencies of their two isomers would be expected. In fact, the *l*-isomer of

FIG. 1. The effect of *d*-isomer of 1-benzyl analogue on cumulative dose-response curve for *l*-isomer.
l-Isomer (—○—) and in the presence of *d*-isomer, 1×10^{-3} Mol (—×—). Each curve is the mean of ten experiments.

the 1-benzyl analogue was about two times as active as the racemate. The *d*-isomer had neither bronchodilating activity nor antagonistic action against the *l*-isomer.

5. Mechanism of action of 1-arylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines

In order to investigate the mechanism of the bronchodilating action, dose-response curves were obtained for the 1-benzyl analogue (VII), 1-(3,4,5-trimethoxybenzyl) analogue (IX), Iso and E in the presence of various concentrations of pronethalol. As shown

FIG. 2. Effects of pronethalol on cumulative dose-response curves for (A) 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (VII), (B) 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (IX), (C) epinephrine, and (D) isoproterenol with 95% confidence limits: control (—○—), and in the presence of pronethalol (—x— 1×10^{-6} Mol; —●— 3×10^{-6} Mol). Each curve is the mean of ten experiments.

in Fig. 2, the higher the concentration of pronethalol, the more shifted to the right were the sigmoid curves of Iso and E. Also, in the case of the THI analogues, a similar dose-dependent parallel shift of the bronchodilator dose-response curve was caused by the β -sympatholytic agent. These results suggest that the THI derivatives compete with pronethalol for the so-called β -adrenergic receptor, and therefore they may be classified as a β -sympathomimetic agent.

DISCUSSION

Generally speaking, possible mechanisms for bronchodilating action are as follows 1) β -sympathomimetic action, exemplified by Iso (12–15), 2) papaverine-like action, exemplified by papaverine, and 3) anti-histaminic or anticholinergic action.

The dose-response curves for THI derivatives were shifted to the right in the presence of pronethalol as in the case of the curves for E and Iso (Fig. 2). In addition, the positive inotropic and blood pressure-lowering actions of the THI derivatives are also antagonized by reasonable doses of pronethalol (9).

FIG. 3. Comparison of chemical structure of catecholamines with that of isoquinoline derivatives.

On the other hand, the so-called papaverine-like action may take part in the bronchodilating action of the present drugs, since the chemical structure of the THI derivatives is partly similar not only to that of catecholamines, but also to that of papaverine (Fig. 3). As anticipated a weak papaverine-like action was revealed using the so-called potassium-depolarized smooth muscle preparation, and the potency varied in parallel with the likeness of the structure of the THI derivatives to that of papaverine. (These results will be published elsewhere in detail by Harigaya in our laboratory.) However, in view of the weakness of their papaverine-like action, it could be concluded that, in comparison with β -sympathomimetic action, it would contribute little to the bronchodilating action.

The other possibility for the mechanism of the drug action, antagonism to histamine or acetylcholine, was discarded, since, within the limit of experimental errors, no difference could be detected between the pD_2 -values for the preparation previously contracted by histamine or acetylcholine and for the untreated control. The control preparation was not relaxed by an anti-histaminic agent, chlorpheniramine, or an anti-cholinergic drug, atropine. (These results will be published elsewhere in detail (10).)

So far as these results indicate, it seems reasonable to conclude that the THI derivatives should be classified as β -sympathomimetics. Holtz reached the same conclusion from his experiments concerning the mode of action of 1-(3,4-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, a member of the series of our THI derivatives (4).

The structure-activity relationship of catecholamines, the representative β -adrenergic stimulants, has been studied for many years by several investigators (11–13, 18–27). Based on the results of these investigations, it is generally accepted that the catecholic hydroxyl groups at position 3 and 4 are essential for manifestation of the β -adrenergic activity (11–13). Pratesi and his co-workers reported that the hydroxyl group at position 3 was more important for the activity than the one at position 4 in the case of epinephrine derivatives (13).

Roughly speaking, the β -mimetic activity of catecholamine derivatives increased in proportion with the size the N-substituents (28). For example, when tested with the isolated tracheal muscle preparation of calf, the pD_2 -values of norepinephrine, and its N-methylated (E), ethylated, isopropylated (Iso), and t-butylated analogues were 5.8, 6.7, 7.2, 7.5 and 7.6 respectively (18). It was also reported (22, 27) that N,N-dimethylated norepinephrine maintained the adrenergic activity but at a decidedly lower level, and the N,N,N-trimethylated analogue was practically devoid of the activity. Based on these facts, it is generally accepted that the secondary nitrogen atom carrying a bulky substituent is essential for the activity.

As for the β -alcoholic hydroxyl group in the side chain of catecholamines, it is considered that it might contribute to the formation of drug-receptor complex by forming a hydrogen bond with a certain site of the receptor so as to facilitate the approach of the catechol and nitrogen groups to the receptor site (11, 12).

Concerning the THI derivatives, Holtz (29) recently reported that two structural elements were essential for the β -sympathomimetic activity of THP, i.e., 1-(3,4-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; 1) two phenolic hydroxy groups in 3,4-position, and 2) a "bulky" substituent on the amino group. In his paper it is not clear whether the 3,4-position indicates the 3,4-position of catecholamine or of THI.

Based on our experimental results with the THI derivatives, it can be concluded that the two hydroxyl groups, essential for manifestation of the bronchodilating activity, were not those on the benzyl group at position 1 of the tetrahydroisoquinoline nucleus but those at position 6 and 7 of the nucleus, corresponding to the 3,4-position of the catecholamines. No difference in contribution to the bronchodilating action between the 6- and 7-hydroxyl groups was assessed, because loss of either hydroxyl group resulted in marked decrease in activity to same extent.

As quoted above, it is stated that, in catecholamines, the nitrogen atom has to be secondary and the substituent on it should be "bulky" for the β -mimetic action. Our experimental results indicate that the nitrogen should also be secondary, because the 2-methylated derivative was ineffective. In the case of THI derivatives the 1-C, the corresponding substituent on the nitrogen, takes part of the heterocyclic nucleus formation and the residual atom group is present as a side chain at position 1 of isoquinoline. According to our results this substituent at position 1 should be not only "bulky", but also an arylmethyl group. Moreover, as shown in Table 1, introduction of certain substituents into the aromatic ring of the side chain affect the activity, it is in this way that the 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (IX), about ten times as

active as Iso, was obtained. The results suggest the possibility that a certain structure might exist in the receptor site, that would interact with the aromatic ring which is attached to the tetrahydroisoquinoline nucleus through one methylene group, and this interaction would play an important role in the formation of the drug-receptor complex. The trimethoxylated benzene ring of the compound IX might be suitable for the formation of the complex. This assumption is of great interest in view of the fact that the THI derivatives were found to be effective as β -sympathomimetics, in spite of the absence of a hydroxyl group at the 4-carbon of the isoquinoline nucleus that would correspond to the alcoholic hydroxyl group in the side chain of catecholamines.

These results suggest that, in the molecules of THI derivatives, the aromatic ring of the side chain might play the role of the β -hydroxyl group of the catecholamines.

Based on the structure-activity relationships described above, it is assumed that the tetrahydroisoquinoline derivatives interact with the β -receptor at three points: the catechol group, the nitrogen atom and the aromatic ring of the arylmethyl group at position 1.

It is of great interest that structural elements that have no similarities in the chemical sense are responsible for the third site of action; for example, an alcoholic hydroxyl group in the side chain of catecholamines, and an arylmethyl, or alkyl [as shown in the Craig's paper (8)], group at position 1 of tetrahydroisoquinolines. These findings can not be explained by the Belleau's β -receptor theory in its present form as treated in the recent literature (12), but they would suggest the possibility that some other structures might exist in the receptor site, that would interact with the arylmethyl or alkyl group and contribute to the formation of the drug receptor complex.

SUMMARY

1. The bronchodilating action of tetrahydroisoquinolines and the structure-activity relationships have been investigated.
2. The bronchodilating action may be due to the β -sympathomimetic action, since the dose-response curves for tetrahydroisoquinolines were shifted to the higher concentration in the presence of pronethalol, as were those for epinephrine and isoproterenol.
3. The chemical structure essential for manifestation of the activity should include
 - i) 1,2,3,4-tetrahydroisoquinoline nucleus,
 - ii) two free hydroxyl groups at position 6 and 7,
 - iii) arylmethyl group at position 1.
4. 1-(3, 4, 5-trimethoxybenzyl)-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline was found to be about ten times as active as isoproterenol.
5. Based on the findings as to the structure-activity relationships, the possible structure of the β -receptor was discussed briefly.

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