Biochemical Pharmacology, Vol. 28, pp. 1307–1312.

0006-2952/79/0415-1307 $02.00/0

DIHYDRO- AND TETRAHYDROISOQUINOLINES AS INHIBITORS OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES FROM DOG HEART

STRUCTURE–ACTIVITY RELATIONSHIPS

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(*Received* 5 *June* 1978; *accepted* 11 *October* 1978)

Abstract—A series of nineteen closely related dihydro- and tetrahydroisoquinolines was examined for inhibitory effects on soluble and particulate preparations of cyclic AMP and cyclic GMP phosphodiesterases from dog heart. Dose–response curves for all the compounds were approximately parallel. 6, 7-Dimethoxy-1-[3-(trifluoromethyl)phenyl]-3, 4-dihydroisoquinoline hydrochloride (USV 2776), the most potent inhibitor in this series, was a competitive inhibitor of all preparations tested, with K_i values of 2–3 μM for membrane cyclic AMP and cyclic GMP phosphodiesterases and 1, 10 and 5μM for soluble "low K_m" cyclic AMP-, "high K_m" cyclic AMP- and cyclic GMP phosphodiesterases respectively. 6-Methoxy-7-benzyloxy-1-phenyl-3, 4 dihydroisoquinoline hydrochloride (USV 2469) was about equipotent to USV 2776; both 2776 and 2469 were two to four times more potent than papaverine and 1-methyl-3-isobutylxanthine. Each dihydroisoquinoline was much more potent than its tetrahydroisoquinoline counterpart. Inhibitory potency was influenced by 6, 7-substitutions, e.g. at the C-6 position, benzyloxy ~ methoxy > hydroxy > hydrogen or methyl. These compounds represent a group of phosphodiesterase inhibitors spanning a 3000-fold activity range, which are related to each other by single structural modifications; they are potentially useful in defining the role of phosphodiesterase inhibition in the various pharmacologic effects elicited by isoquinolines, including papaverine.

The isoquinoline moiety is the basic component of a variety of compounds having numerous pharmacological effects such as bronchodilation, vasodilation, spasmolytic activity and antiarrhythmic activity [1–6]. The postulated mechanism of action for many of these compounds has involved the inhibition of cyclic nucleotide phosphodiesterases. However, many of these compounds have been shown to have other biochemical effects, such as alteration of Ca^{2+} fluxes [7, 8], and thus, the actual mechanism of action responsible for any pharmacological response remains in doubt. One of the most well-investigated compounds of this type, whose pharmacological mechanism of action reportedly is via the inhibition of cyclic nucleotide phosphodiesterase but which also alters Ca^{2+} fluxes, is papaverine (6, 7 dimethoxy-1-(3′,4′-dimethoxy-benzyl-isoquinoline). One approach to analyzing the mechanism of action is to study the activities of very closely related analogs as has been done with xanthine derivatives as lipolytic agents [9, 10]. Some fragmentary structure–activity relationships for papaverine and related isoquinoline analogs as phosphodiesterase inhibitors have been described previously in the literature [10–21] and discussed in reviews [22, 23]. However, many of the relationships described were derived from the comparison of activities of structurally related compounds differing from each other by more than one structural feature. Possibly for this reason, there are some differences in the structure–activity relationships reported for these compounds. For example, previous reports have not correlated clearly the degree of saturation of the nitrogen-containing ring with changes in activity, and these reports have suggested, but not clearly demonstrated, that quaternization of this nitrogen is detrimental to activity [22].

The relationships between the structures of several dihydro- and tetrahydroisoquinolines and their abilities to inhibit dog heart cyclic nucleotide phosphodiesterases are described in this paper. These relationships are derived from comparison of the activities of compounds which differ from each other by single chemical modifications. Since these analogs are chemically similar to papaverine, the structure–activity relationships are also compared to those reported for papaverine analogs. The series of compounds reported here has potential utility for correlating the inhibition of cyclic nucleotide phosphodiesterase with pharmacological effects.

MATERIALS AND METHODS

Cyclic nucleotide phosphodiesterase was assayed by a modification of the "one-step" assay described by Thomson *et al.* [24], with the entire assay being done in small (7 ml) scintillation vials using an 0.2 ml assay volume. As quantitated with ^{14}C-nucleotides (New England Nuclear, Boston, MA) the recovery of assay products was approximately 80–90 per cent for adenosine and 75–80 per cent for guanosine with 0.01 to 1 μM nucleotide. Separation of the enzyme products from their substrates was approximately 94–97 per

cent complete. Test compounds were solubilized in either assay buffer or ethyl alcohol at a final concentration of 1.0% (v/v). This concentration of alcohol had no measurable effect on the V_{max} or K_m values of the enzymes measured nor did it measurably affect the I_{50} values of commonly used inhibitors such as theophylline and 1-methyl-3-isobutylxanthine.

The crude enzyme fractions were prepared as follows. Mongrel male dogs were anesthetized with Nembutal (35 mg/kg) and their hearts were removed surgically and rinsed in 0.15 M NaCl (4°). The ventricular muscle was cut into large pieces and homogenized with a prechilled Waring blender (medium speed for 30 sec) in 3 vol. of 0.25 M sucrose and 50 mM Tris–Cl, pH 7.4 (4°). This homogenate was strained through two layers of cheesecloth and then centrifuged at 105,000 *g* for 90 min (30,000 rev/min, Type 30 Beckman rotor). The resulting supernatant fraction was used as the "soluble" enzyme preparation. The resulting pellet was washed by resuspension in homogenization buffer and recentrifugation. This washed pellet was resuspended and used as the "membrane" enzyme preparation. Both enzyme preparations were stored in small aliquots at −80°. Just prior to use, enzyme aliquots were thawed and diluted in assay buffer to the appropriate concentration for linear assay conditions. Usually, 5–50 μg of membrane protein or 1–10 μg of soluble protein was added, depending on the type and the concentration of substrate used. All dihydro- and tetrahydroisoquinoline derivatives were synthesized and verified for purity and chemical composition as described elsewhere [25]. Nucleotides and nucleosides were purchased from Boehringer Mannheim, Indianapolis, IN. Tritium-labeled cyclic nucleotides were obtained from New England Nuclear and purified as described [24]. All other chemicals were reagent grade. Proteins were determined as described [26].

RESULTS

Kinetic analysis of the enzyme preparations indicated that the membrane preparation hydrolyzed cyclic AMP with a V_{max} of about 2.5 nmoles/min/mg of protein and cyclic GMP with a V_{max} of about 1.0 nmole/min/mg of protein. The apparent Michaelis–Menten constants (K_m) were 1–2 μM for cyclic AMP and 1–2 μM for cyclic GMP. The double reciprocal plot for cyclic AMP hydrolysis by the membrane preparation indicated the usually described anomalous behavior at high concentrations [27, 28] which is indicative of either site to site interaction on one type of enzyme or the presence of a second type of enzyme having a relatively high K_m, i.e. 10 μM. The soluble enzyme preparation hydrolyzed cyclic GMP with a V_{max} of about 4 nmoles/min/mg of protein and an apparent K_m of 2 μM. The kinetic profile for the hydrolysis of cyclic AMP by the soluble preparation indicated the presence of multiple catalytic sites with extrapolated K_m values of 2 μM and 15 μM and associated V_{max} values of 10 and 25 nmoles/min/mg of protein. These values are consistent with those reported for heart enzymes [29, 30]. For this work the "low K_m" and "high K_m" soluble cyclic AMP phosphodiesterases were distinguished solely on the basis of hydrolysis at a low (0.5 μM) and at a high (20 μM) substrate concentration respectively.

The dose–response curves for the inhibitory effects of papaverine and the dihydro- and tetrahydroisoquinoline derivatives tested were all approximately parallel to each other for all substrates and enzymes measured. As an example of the parallel dose–response curves, the effects on cyclic GMP phosphodiesterase from the membrane preparation are illustrated in Fig. 1. Dixon plot analysis [31] and double-reciprocal plot analysis indicated that USV 2776, the most potent analog of this series, was a competitive inhibitor of all the enzyme forms tested. Again as an example, the data with cyclic GMP phosphodiesterase from the membrane preparation are illustrated in Fig. 2. The inhibitory constants (K_i) for USV 2776 were 2–3 μM for both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase from the membranes, and were 1, 10 and 5 μM for the soluble "low K_m" cyclic AMP phosphodiesterase, "high K_m" cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase respectively. These values

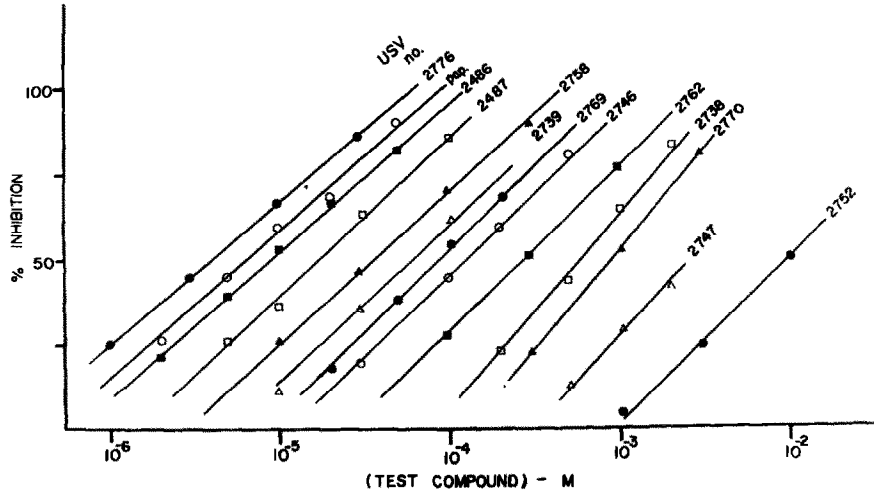


Fig. 1. Dose–response curves of the effects of isoquinoline analogs on cyclic GMP phosphodiesterase of pellet fraction of dog heart. Cyclic GMP phosphodiesterase was assayed at 0.5 μM substrate in the presence of the indicated compounds; see Table 1 for their structures.

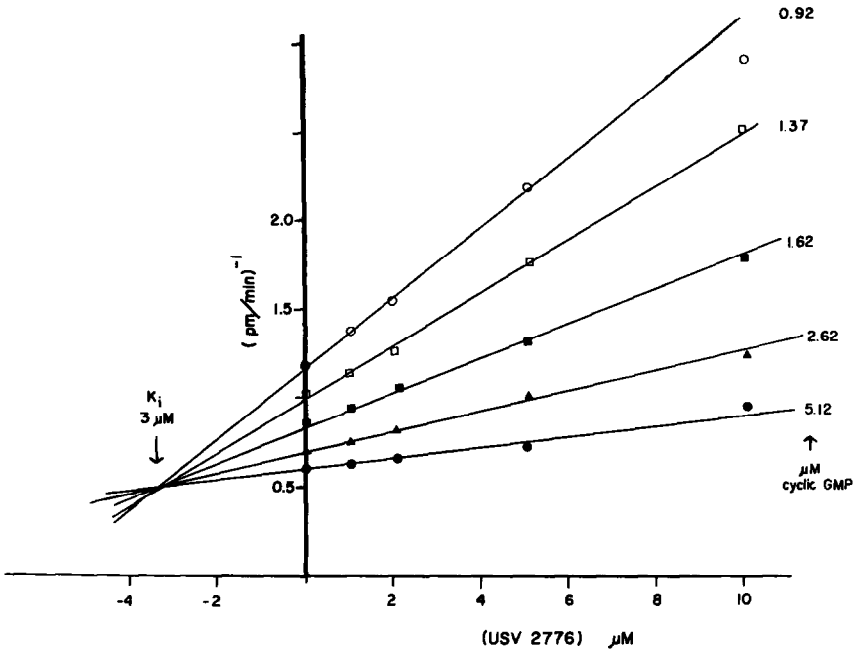
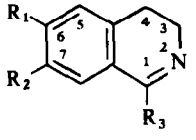
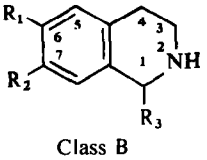


Fig. 2. Dixon plot analysis of the effects of USV 2776 on cyclic GMP phosphodiesterase from dog heart pellet.

Table 1. Structures of dihydroisoquinolines (class A) and tetrahydroisoquinolines (class B) and their effective I_{50} values against dog heart cyclic nucleotide phosphodiesterases



Class A



Class B

Compound No.	USV I.D. No.	R_1	R_2	R_3	I_{50} (μM) Pellet prep.* cAMP⁺ (0.5 μM)	Pellet prep.* cGMP⁺ (0.5 μM)	Soluble prep.* cAMP⁺ (0.5 μM)	Soluble prep.* cAMP⁺ (20 μM)	Soluble prep.* cGMP⁺ (5 μM)
Dihydroisoquinolines (Class A)									
1‡	2486	H_3CO	H_3CO	C_6H_5	10	9	20	100	50
2‡	2469	H_3CO	$C_6H_5CH_2O$	C_6H_5	5	4	5	30	15
3‡	2487	H_3CO	HO	C_6H_5	40	25	40	300	100
4‡	2746	H	H	C_6H_5	200	150	200	1,000	400
5	2771	H	H_3C	C_6H_5	100	100	80	1,000	200
6	2776§	H_3CO	H_3CO	m-CF_3-C_6H_4	4	6	3	25	30
7	2769	H_3CO	H_3CO	o-CH_3O-C_6H_4	35	60	30	200	80
8‡	2735	H_3CO	H_3CO	C_6H_{11}	50	35	40	300	300
Tetrahydroisoquinolines (Class B)									
9‡	2610	H_3CO	H_3CO	C_6H_5	200	200	200	1,500	700
10‡	2470	H_3CO	$C_6H_5CH_2O$	C_6H_5	150	100	100	—	—
11‡	2621	H_3CO	HO	C_6H_5	1,000	700	1,000	5,000	3,000
12‡	2747	H	H	C_6H_5	2,500	2,500	3,000	20,000	5,000
13	2763	H	H_3C	C_6H_5	4,000	5,000	3,000	⩾ 10,000	⩾ 10,000
14	2758	H_3CO	H_3CO	m-CF_3-C_6H_4	30	30	25	300	100
15	2762	H_3CO	H_3CO	o-CH_3O-C_6H_4	150	250	125	—	400
16‡	2738	H_3CO	H_3CO	C_6H_{11}	650	650	650	3,000	2,500
17	2770	H	H_3CO	C_6H_5	800	800	1,000	5,000	2,500
18	2752	H	H	H	5,000	10,000	10,000	⩾ 20,000	⩾ 20,000
19‡	2739	H_3CO	H_3CO	p-$(CH_3)_3C$-C_6H_4	40	45	45	300	300
Standard inhibitors									
20	Papaverine§				8	16	19	105	85
21	1-Methyl-3-isobutylxanthine				10	10	20	200	60
22	Theophylline				130	150	190	1,000	500

* Enzyme preparation.
⁺ Nucleotide substrate.
‡ Tested at ⩾ 2 times the highest I_{50} concentration for effects on 5′-nucleotidase.
§ I_{50} values are averages of five separate experiments.

Table 2. Comparative results of the most active inhibitors

Compound	N*	I_{50} values ± S.E.M. Pellet† cAMP‡ (0.5 μM)	Pellet† cGMP‡ (0.5 μM)	Soluble prep.† cAMP‡ (0.5 μM)	Soluble prep.† cAMP‡ (20 μM)	Soluble prep.† cGMP‡ (5 μM)
Papaverine	5	8 ± 2	16 ± 1	19 ± 6	105 ± 30	85 ± 7
MIX§	3	12 ± 1	19 ± 3	17 ± 6	180 ± 15	85 ± 30
USV 2776	5	4 ± 1	6 ± 2	3 ± 1	25 ± 4	30 ± 10
USV 2469	3	5 ± 2	7 ± 5	4 ± 1	25 ± 4	14 ± 3

* Number of separate dose–response curves used to determine I_{50} values.
† Enzyme fraction.
‡ Substrate.
§ MIX = 1-methyl-3-isobutylxanthine.

agree favorably with those derived from the values as calculated for competitive inhibitors, i.e. $I_{50} = K_i (1 + S/K_m)$ [32]. Since the inhibitory concentration–response curves for all the isoquinolines were parallel, K_i values were not determined for all of the compounds tested, and the I_{50} values were used for the determination of the structure–activity relationships.

Table 1 lists the structures of all the compounds tested and their measured I_{50} values against cyclic nucleotide phosphodiesterases. All the compounds were tested at one concentration in at least two experiments prior to being tested at four or more concentrations, which caused 25–80 per cent inhibition, to determine I_{50} values. To evaluate repeatability further, several compounds were retested at multiple concentrations with more than one heart preparation. The data in Table 2 exemplify the type of repeatability obtained.

Within experimental error, the rank order of potencies of each of these isoquinoline analogs relative to papaverine and USV 2776 was similar for each of the different forms of enzymes measured. Thus, none of these compounds showed striking specificity for one enzyme form. These differences in the I_{50} values on the different enzyme forms for each of these compounds appeared to be more of a function of the K_m values for substrate rather than the type of cyclic nucleotide hydrolyzed or the fraction of the tissue from which the enzyme was measured. Both 2776 and 2469 were about two to four times more potent than papaverine and 1-methyl-3-isobutylxanthine (Table 2).

Several of these isoquinoline analogs (see Table 1) were tested for their effects on the 5′-nucleotidase activity in snake venom (*Ophiophagus hannah*), since inhibition of this enzyme would erroneously suggest inhibition of cyclic nucleotide phosphodiesterase. At concentrations causing about 100 per cent inhibition of cyclic nucleotide phosphodiesterase, none of the isoquinolines tested had any measurable effect on the snake venom 5′-nucleotidase. This 5′-nucleotidase activity was measured with the assay conditions for cyclic nucleotide phosphodiesterase, using tritiated 5′-AMP as the substrate.

When the I_{50} values were compared for eight separate pairs of compounds in which each member of the pair differed only by the saturation of the bond between the C-1 and N atoms (Compounds 1–8 vs 9–16, Table 1), the dihydroisoquinolines were 16 ± 4 (2 S.E.M.) times more potent than the tetrahydroisoquinolines. This potency difference, due to the saturation of the bond, was about the same for all enzyme preparations measured.

On the basis of comparing I_{50} values from at least two separate pairs of compounds in which each member of the pair differs by a single chemical parameter, the following structure–activity relationships were observed for changes at the C-7 position. In comparison to the compounds with a methoxy group (compounds 1 and 9), those with the benzoyl ester were about two to three times more potent (compounds 2 and 10), whereas those with a hydroxyl group were about three to five times less potent (compounds 3 and 11). Compounds with a hydrogen were about equally potent to those with a methyl group (compounds 4 and 12 vs 5 and 13).

Little can be inferred from this series about substitutions at the C-6 locus except that the compound with a methoxy group is about five times more potent than the compound with a hydrogen (compound 9 vs 17). The substitution of both methoxy groups at C-6 and C-7 with hydrogens resulted in about a 15-fold loss of potency (compounds 1 and 9 vs 4 and 12). This might suggest that changing the methoxy group at C-7 to a hydrogen caused about a 3-fold decrease in potency.

With comparable compounds the following structure–activity relationships were observed with changes at the C-1 position. In comparison to the compounds with a phenyl group (compounds 1 and 9), those with a cyclohexyl group were at least two times less potent (compounds 8 and 16), whereas those with a 3′-trifluoromethyl-phenyl group (compounds 6 and 14) were at least two times more potent. The addition of the 2′ methoxy group to the 1-phenyl group (compounds 7 and 15) caused about a 2-fold decrease in potency with the dihydro analog (compound 1), but had little effect with the tetrahydro analog (compound 9).

The compound with a hydrogen at the C-1 position was about 2- to 4-fold less potent than a comparable compound with a phenyl group (compound 12 vs 18). The compound with a 4′-butyl-phenyl at the 1-carbon was about two to three times more potent than one with a phenyl (compound 19 vs 9).

With this series of isoquinolines, quaternization of the nitrogen did not seem to alter activity. For example, the methiodide salt of USV 2486 was equipotent to USV 2486.

DISCUSSION

The dihydro- and tetrahydroisoquinoline analogs described in this paper show some interesting and clear-cut relationships between the chemical modifications of the basic isoquinoline moiety and the ability to inhibit cyclic nucleotide phosphodiesterases. These isoquinoline analogs appear to be competitive inhibitors of all forms of cyclic nucleotide phosphodiesterase from dog heart. The structure–activity relationships are based on the comparative potencies of pairs of analogs in which members of the pair differ from each other by single chemical modifications. The structure–activity relationships for these dihydro- and tetrahydroisoquinolines have many similarities to those described in the literature for papaverine analogs. For example, alteration of the C-6 and C-7 methoxy groups drastically affects inhibitory activity, as does altering the substituent at the C-1 position. The following structure–activity relationships of the dihydro- and tetrahydroisoquinolines may also apply to activity relationships for papaverine analogs. Saturation of the 2–3 bond caused a 16-fold reduction in potency as a cyclic nucleotide phosphodiesterase inhibitor. Quaternization of the nitrogen at the 2 position does not necessarily affect potency. Introduction of a trifluoro-methyl group at the 3′ position of the 1-phenyl group enhanced activity, as did introduction of a tertiary butyl group at the 4′ position of the 1 phenyl group. Methoxy groups impart more potency at the C-6 and C-7 positions than hydroxyl, hydrogen, or methyl groups.

It is interesting to note that the difference in potency due to multiple substitutions could be ascribed in several cases to a multiple of the differences due to each of the individual changes. For example, USV 2486 and USV 2621 differ at the C-6 (hydroxy vs methoxy) and in the saturation of the C-1 and N bond. Based on the above generalizations about the structure–activity relationships, changing the methoxy group to a hydroxy group should have decreased the potency about four times, and saturation of the 1-2 double bond should have decreased potency about sixteen times. Thus, we would have expected a 4×16 or a 64-fold change in potency due to the two changes. The measured difference in potency was about 68-fold. This type of example suggests that caution should be used in assuming structure–activity relationships between compounds with more than one chemical modification. Certainly, misleading inferences could be drawn from comparing compounds with multiple modifications, particularly if one change enhanced activity and one decreased activity. In this regard, the expected difference in potency between USV 2486 and USV 2578, based on the above generalization, would be 4- to 5-fold, i.e. a 3-to 4-fold increase for addition of the CF_3 groups and a 16-fold decrease for the saturation of the C 1-2 bond. The actual measured difference was about 3-fold.

Since multiple substitutions can result in additive or conflicting changes in potency, direct comparisons of the potencies of the 1-phenylisoquinoline analogs described in this paper with those described in the literature are difficult, since the compounds in the literature differ from those described in this paper by two or more structural features [22, 23]. Furthermore, almost all of the isoquinoline analogs described in the literature as inhibitors of cyclic nucleotide phosphodiesterase contain a benzyl group at the C-1 position and this benzene ring would be expected to assume a different configuration, relative to the isoquinoline nucleus, than the benzene ring in the 1-phenyl derivatives. Since USV 2776 and papaverine inhibited cyclic nucleotide phosphodiesterase in a competitive fashion and since the tetrahydroisoquinolines and other dihydroisoquinolines gave inhibition–concentration plots parallel to that of USV 2776, all three classes of isoquinolines probably interact with the enzyme site that binds substrate. However, it is possible that the phenyl derivatives assume a slightly different orientation with the catalytic site on the enzyme than do the benzyl congeners.

Since the cyclic nucleotide phosphodiesterase inhibitory activity of this series of isoquinoline analogs can be related to single chemical modifications and since these compounds show large differences in their potency, this series has the potential for use in correlating inhibition of cyclic nucleotide phosphodiesterase with pharmacological effects for isoquinolines in general, and, by analogy for papaverine in particular. Thus, it may be possible to determine to what extent the inhibition of cyclic nucleotide phosphodiesterases is involved in pharmacologic responses to isoquinolines and to what extent other mechanisms, such as modulation of Ca^{2+} fluxes, are involved.

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