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DOPAMINE-DERIVED TETRAHYDROISOQUINOLINE ALKALOIDS—INHIBITORS OF NEUROAMINE METABOLISM*

ALLAN C. COLLINS,† JESSE L. CASHAW and VIRGINIA E. DAVIS

Neurochemistry and Addiction Research Laboratory, Veterans Administration Hospital, Houston, Texas, U.S.A.

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Abstract—The formation of salsolinol and tetrahydropapaveroline (THP) *in vitro* as aberrant metabolites of dopamine has been established previously. Demonstrating the formation of these compounds *in vivo* is complicated by the probability that they are extensively metabolized. The present studies indicate that a primary metabolic route for these compounds may be methylation of one or more of the free hydroxyl groups catalyzed by catechol-*O*-methyl transferase (COMT). Through the use of a partially purified COMT preparation from rat liver, the maximal velocities of salsolinol and THP *O*-methylation proved to be three to five times the maximal velocities of norepinephrine and dopamine *O*-methylation. The Michaelis constants determined for salsolinol, norepinephrine and dopamine are similar, whereas the K_m of COMT for THP (0·03 mM) is approximately one-tenth that of the above substrates. Salsolinol and THP are competitive inhibitors of dopamine *O*-methylation *in vitro*, their calculated inhibitor constants (K_i values) being 0·13 and 0·02 mM respectively. The effects of these two alkaloids on rat brain monoamine oxidase (MAO) activity were also measured. They were found to be equally potent substrate competitive inhibitors of rat brain MAO *in vitro*, with calculated K_i values of 0·14 mM (salsolinol) and 0·20 mM (THP). These data suggest that, if formed *in vivo* under certain pharmacological conditions, aberrant neuroamine-derived alkaloids may alter the metabolic disposition of endogenous neuroamines with resultant modification of adrenergic function.

THE FORMATION of two dopamine-derived tetrahydroisoquinoline alkaloids has been demonstrated *in vitro*.[1–3] The simpler of the two is 1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline (salsolinol), which is formed by the direct condensation (via a Schiff's base intermediate) of acetaldehyde and dopamine.[1] A more complex alkaloid, 1,2,3,4-tetrahydro-6,7-dihydroxy-1-(3′,4′-dihydroxybenzyl)-isoquinoline (tetrahydropapaveroline, THP, norlaudanosoline), is formed by a similar condensation of dopamine and 3,4-dihydroxyphenylacetaldehyde. This last compound is produced by the action of monoamine oxidase (MAO) on dopamine (Fig. 1). Acetaldehyde, the primary metabolite of ethanol, is a requisite precursor of salsolinol. It is also capable of decreasing the oxidative metabolism rate of 3,4-dihydroxyphenylacetaldehyde. Hence the endogenous formation of both these alkaloids might be expected to increase after consumption of ethanol.[3]

Both the simple 1-methyltetrahydroisoquinoline alkaloids related to salsolinol[4–7]

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† Present address: School of Pharmacy, University of Colorado, Boulder, Colo.

and the more complex alkaloids related to THP[8–13] are pharmacologically active. The acute pharmacological effects of THP, which are similar to those of isoproterenol, have been well documented. THP produces positive inotropic and chronotropic responses in heart preparations. It generates hypotensive effects and increases blood flow in intact animals, relaxation of isolated smooth muscle preparations, and mobilization of lipids.[8–13] All these actions are antagonized by β-adrenergic blocking agents (such as propranolol, dichloroisoproterenol and pronethalol), but are not abolished by prior reserpinization of the animals.

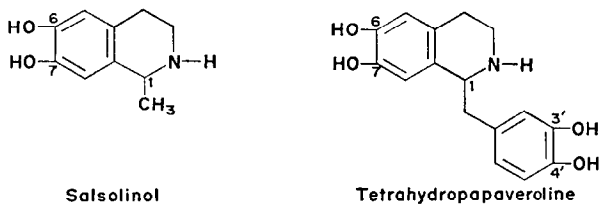

FIG. 1. Dopamine-derived tetrahydroisoquinoline alkaloids. Salsolinol [1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline] is formed by the condensation of acetaldehyde with dopamine. Tetrahydropapaveroline [1,2,3,4-tetrahydro-6,7-dihydroxy-1-(3′,4′-dihydroxybenzyl)-isoquinoline, norlaudanosoline, THP] is formed by condensation of the aldehyde derivative of dopamine, 3,4-dihydroxyphenylacetaldehyde, with the parent amine.

The endogenous production of salsolinol and THP *in vivo* may play a role in evoking some of the pharmacological effects of the drugs that enhance their formation.[1,3,14,15] The credibility of this suggestion is strengthened by the recent unequivocal demonstration of the formation of both salsolinol and tetrahydropapaveroline *in vivo* in man.[16] Determination of the total production of these tetrahydroisoquinoline alkaloids *in vivo* is complicated by the probability that they are extensively metabolized. Both alkaloids retain vicinal hydroxyl groups on an aromatic nucleus. It therefore seems reasonable to assume that at least one metabolic pathway of these compounds in mammals may be methylation by catechol-*O*-methyl transferase (COMT). In this paper norepinephrine, dopamine, salsolinol and THP are compared as substrates for a partially purified rat liver COMT preparation. Evidence is presented regarding the nature of the *O*-methylated products of salsolinol and THP, together with data demonstrating that both alkaloids are inhibitors of mammalian COMT *in vitro*. In addition, their effect on MAO activity is described.

MATERIALS AND METHODS

Compounds used. Radiochemicals were purchased from the following suppliers: [7-^{3}H]normetanephrine, [5-^{3}H]3-methoxy-4-hydroxy-β-phenylethylamine (3-methoxytyramine) and [2-^{3}H]3,4-dihydroxyphenylethylamine (dopamine) came from New England Nuclear Corp., Boston, Mass.; *S*-adenosyl-L-methionine-[methyl-^{14}C] and 5-hydroxytryptamine-[3′-^{14}C]-creatinine sulfate (serotonin) from Amersham/Searle, Arlington Heights, Ill. Salsolinol hydrobromide, laudanosoline hydrobromide trihydrate, isoquinoline, tetrahydroisoquinoline, salsolidine hydrochloride dihydrate and laudanosine were purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Serotonin creatinine sulfate complex (5-hydroxytryptamine), *S*-adenosyl-L-methionine iodide hexahydrate, 3-hydroxytyramine hydrochloride (dopamine), and DL-arterenol

hydrochloride (norepinephrine) were obtained from CalBiochem, La Jolla, Calif. Triton X-100 was purchased from Rohm & Haas Co., Philadelphia, Pa.

The alkaloids, 1-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline, 1-(3′,4′-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide (tetrahydropapaveroline, THP, norlaudanosoline), and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (norlaudanosine) were a gift of the Wellcome Research Laboratories, Beckenham, Kent, England.

Reticuline, norreticuline, orientaline and nororientaline were kindly provided by Dr. E. Brochmann-Hanssen of the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, Calif.

The 6-methoxy derivative of salsolinol [1,2,3,4-tetrahydro-6-methoxy-7-hydroxy-1-methylisoquinoline] was prepared from 3-methoxytyramine and acetaldehyde using the conditions described by Schopf and Bayerle[17] for the synthesis of salsolinol.

Bis-trimethylsilyltrifluoroacetamide/1% trimethylchlorosilane (BSTFA/1% TMCS) was purchased from Regis Chemical Co., Chicago, Ill.

Relative activity of substrates for COMT. Partially purified COMT was obtained from the livers of adult male Sprague–Dawley (TEX) rats according to the method of Axelrod and Tomchick,[18] except that the soluble supernatant fraction was prepared by 60-min centrifugation at 27,000 *g*.

Through a modification of the assay method of Creveling *et al.*,[19] the catecholamines and tetrahydroisoquinoline alkaloids were compared as substrates for rat liver COMT. Various amines and alkaloids were also tested as substrates for rat brain COMT. The high-speed supernatant of 20 per cent brain homogenates was the enzyme source.

Varying concentrations of substrates were incubated with 0·6 μmole of $MgCl_2$, 0·25 μmole of *S*-adenosyl-L-methionine (SAM), 0·035 μCi of [^{14}C] SAM, 50 μmoles of pH 8·0 phosphate buffer, 0·25 mg of ascorbic acid, and enzyme preparation (approximately 1 mg of protein) in a final incubation volume of 0·5 ml. The reaction mixtures were incubated at 37·5° for 60 min. They were terminated by adding 0·5 ml of 0·5 M, pH 10 borate buffer; or, in the case of the THP incubations, 0·5 ml of 0·5 M, pH 6·0 phosphate buffer and immediately the methylated reaction products were extracted into 5 ml of toluene–isoamyl alcohol (3:2). This minor modification was necessary because the methylated products of dopamine, norepinephrine and salsolinol are maximally extracted at pH 10·0, whereas optimum extraction of the methylated products of THP is obtained at pH 6·0. To measure radioactivity, a 4·0-ml aliquot of the organic layer was removed and added to 15 ml of toluene–Triton X-100 scintillation fluid.[2] Under these incubation conditions, the reactions were linear with time and protein concentration for at least 60 min.

Commercially available radiolabeled normetanephrine and 3-methoxytyramine were used to determine extraction efficiency of *O*-methylated products of norepinephrine and dopamine respectively. The reaction products of salsolinol and THP with COMT and SAM are not available commercially. The extraction efficiencies of these methylated alkaloid derivatives were therefore estimated by evaporating aliquots of the toluene-isoamyl alcohol extracts of incubation mixtures and then re-extracting these enzymatically synthesized radioactive reaction products. Extraction efficiency ranged between 65 and 80 per cent and data were corrected for recovery and counting efficiency.

Inhibition of dopamine O-methylation. In experiments to assess the inhibition of dopamine *O*-methylation *in vitro* by salsolinol, THP and other tetrahydroisoquinoline alkaloids, [^{3}H]dopamine (0·16 μCi) replaced [^{14}C]SAM as the tracer. Consequently the degree of the potential inhibitor's *O*-methylation was not a factor in calculating the rate of dopamine *O*-methylation.

Inhibition of MAO. Salsolinol's and THP's potential inhibition of rat brain MAO activity was determined by using a modification of the assay method of Robinson *et al.*[20] Serotonin (0·05–1·0 mM), containing 0·035 μCi [^{14}C]-5-hydroxytryptamine as a tracer, was added to the specified amounts of salsolinol or THP and incubated for 30 min at 37·5° with 0·05 ml of 10 per cent rat brain homogenate in 0·2 M phosphate buffer, pH 7·5. Total volume of the reaction mixture was 0·5 ml. Metabolic activity was halted by placing the samples in boiling water for 3 min.

The denatured protein was removed by centrifugation. The supernatants were transferred directly onto 0·5 × 2·5 cm columns of Amberlite CG-50 Na^+ form, 200–400 mesh, pH 6·5, ion-exchange resin. The protein precipitates were once again suspended in 1·0 ml of water and centrifuged, the supernatants thus produced being transferred to the ion-exchange columns.

After the samples passed through the columns, the columns were washed with an additional 3·0 ml of water. The wash completely removed the serotonin metabolites; the serotonin remained adsorbed on the resin. The effluent and wash containing the reaction products were combined and diluted to 10 ml. A 2·0-ml aliquot was removed, added to 15 ml of toluene–Triton X-100 scintillation fluid, and assayed for radioactivity. The rate at which deamination products of serotonin formed in the presence and absence of both salsolinol and THP was then observed.

Blank values for all enzymatic assays were obtained by substituting a boiled enzyme preparation for the active enzyme in the incubation mixtures. Protein was measured by the biuret method.[21]

Gas chromatography. To identify the *O*-methylated reaction products of salsolinol and THP, analyses were performed with a Barber–Coleman (Series 5000) gas–liquid chromatograph equipped with a flame ionization detector. Gas chromatograph conditions were: 6-ft U-shaped glass column (i.d., 4 mm) packed with 3% OV-1 on Gas Chrom Q, 100–120 mesh, detector temperature 290°, and nitrogen flow rate, 60 ml/min. The instrument was fitted with a stream splitter (50:50), so that radioactivity of each peak could also be monitored. The methylated products of salsolinol were analyzed under isothermal conditions at 190°, and of THP, at 260°.

The methylated reaction products of salsolinol and THP were extracted into toluene–isoamyl alcohol and were extracted with one-tenth volume of 0·1 N HCl. The acid extract was dried under vacuum for 2 hr. The dried extract was reacted with BSTFA/1% TMCS (10 μl) in acetonitrile (90 μl) at 65° for 3·5 hr to form trimethylsilyl (TMSi) derivatives of the methylated reaction products for gas chromatographic analysis. Authentic standards (1 μg/μl) were prepared in methanol and an aliquot (20 μl) of each reference standard solution was dried and reacted with BSTFA/1% TMCS in a similar manner. Generally, 2–6 μl of these reaction mixtures was used to determine the retention time.

RESULTS

Dopamine-derived alkaloids as substrates for COMT. A comparison of the relative

effectiveness of norepinephrine, dopamine, salsolinol and THP as substrates for rat liver COMT is graphed in Fig. 2. The Lineweaver–Burk plot obtained from kinetic analyses reveals that norepinephrine, dopamine and salsolinol have nearly identical apparent K_m values (0·66, 0·23 and 0·29 mM). With THP as substrate, the apparent K_m value (0·03 mM) is approximately 10 per cent that of dopamine. In addition, the maximal velocities of both salsolinol and THP *O*-methylation are three and five times greater, respectively, than the maximal velocity obtained with dopamine (Table 1). The relatively low reaction velocities and the extended period over which linearity with time was achieved indicate that an enzyme with low specific activity had been obtained in the purification procedure. The kinetic analyses would appear to be valid, however, since even at the lowest substrate concentrations only a small fraction of substrate had been utilized.

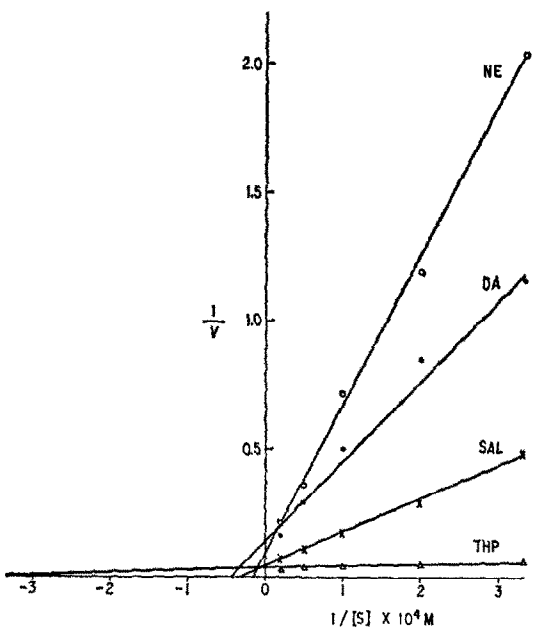

FIG. 2. Lineweaver–Burk plot comparing norepinephrine (NE), dopamine (DA), salsolinol (SAL) and tetrahydropapaveroline (THP) as substrates for rat liver catechol-*O*-methyl transferase. The assay system was the same as given in Table 1. Substrate concentrations ranged between 0·03 and 0·5 mM.

It should be noted that these data are derived on the basis of a mole per mole relationship between substrate and *S*-adenosyl-L-methionine. However, the THP molecule contains two moieties with catechol functional groups and it is possible that methylation of THP may occur at the 6, 7, 3' or 4' position or at more than one of these positions. Thus, precise interpretation of the kinetic data obtained with THP will require identification of the *O*-methylated products formed.

The alkaloids were also tested as substrates for rat brain COMT. Similar K_m and maximal velocity values were obtained when rat brain preparations were substituted for the partially purified rat liver preparations of COMT. These data suggest that

not only are both of the dopamine-derived tetrahydroisoquinoline alkaloids substrates for COMT, but also that they might be inhibitors of catecholamine *O*-methylation.

TABLE 1. MICHAELIS CONSTANTS AND MAXIMAL VELOCITIES OF RAT LIVER COMT FOR CATECHOLAMINES AND DOPAMINE-DERIVED ALKALOIDS*

Substrate	K_m (mM)	V_{max}
Dopamine	0·23	7·04
Norepinephrine	0·66	11·36
Salsolinol	0·29	22·73
Tetrahydropapaveroline	0·03	37·04

* The K_m values for the amines and alkaloids were determined in reaction mixtures containing varying concentrations of the substrates, 0·6 μmole of $MgCl_2$, 0·25 μmole of SAM, 0·035 μCi of [^{14}C]SAM, enzyme preparation (approximately 1 mg of protein) and 50 μmole of sodium phosphate, pH 8·0. The final volume was 0·5 ml and the systems were incubated at 37·5° for 60 min. Michaelis constants and maximal velocities were determined by the conventional Lineweaver–Burk double reciprocal plot. V_{max} values are expressed as nmoles of *O*-methylated product formed/mg of protein per hr.

Competitive inhibition of catecholamine O-methylation. In Fig. 3. is a graphic analysis of the experiments to determine salsolinol's potential inhibition of dopamine *O*-methylation. The Lineweaver–Burk plot produced a pattern typical of a competitive inhibitor. The calculated K_i of 0·13 mM is in the same range as the K_m of COMT for salsolinol (0·29 mM).

The results of the experiments testing THP as an inhibitor of dopamine *O*-methylation establish yet another pattern of competitive inhibition (Fig. 4). The THP concentrations used were one-tenth those of the salsolinol used in the inhibition experiments reported in Fig. 3, and the calculated K_i of 0·02 mM was nearly one-tenth the K_i value of salsolinol. Thus, as would be expected from a comparison of apparent K_m values, THP has approximately ten times the potency of salsolinol as a dopamine *O*-methylation inhibitor.

Isoquinoline and tetrahydroisoquinoline (which contain no hydroxy groups) and various completely *O*-methylated tetrahydroisoquinoline alkaloids (such as salsolidine, norlaudanosine and laudanosine), structurally related to either salsolinol or THP, were found to have no effect on the rate of dopamine *O*-methylation *in vitro*. Clearly free hydroxyl groups are requisite for these compounds to act as competitive inhibitors of catecholamine *O*-methylation.

Inhibition of MAO. Both salsolinol and THP are inhibitors of serotonin oxidation by MAO *in vitro* (Figs. 5 and 6). The inhibition pattern demonstrated by both compounds is characteristic of a substrate competitive inhibitor. The calculated inhibition constants are 0·14 mM for salsolinol and 0·20 mM for THP. As inhibitors of rat brain MAO activity, therefore, these compounds are almost equally effective.

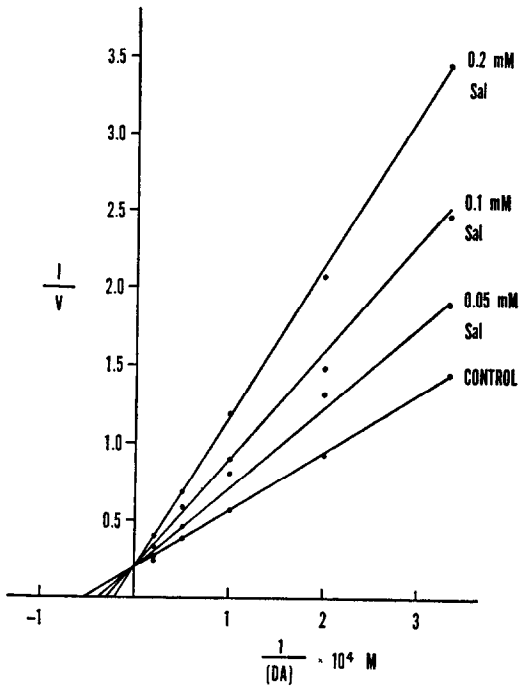

FIG. 3. Lineweaver–Burk plot illustrating the competitive inhibition of dopamine *O*-methylation by salsolinol (SAL) (0·05, 0·1 and 0·2 mM). K_i = 0·13 mM.

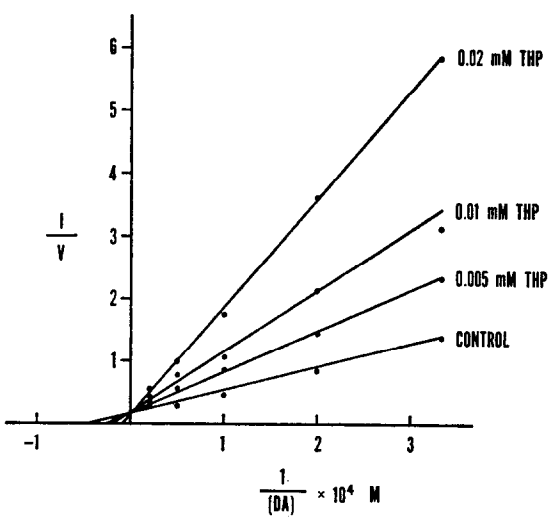

FIG. 4. Lineweaver–Burk plot of the competitive inhibitory effect of tetrahydropapaveroline (THP) (0·005, 0·01 and 0·02 mM). K_i = 0·02 mM.

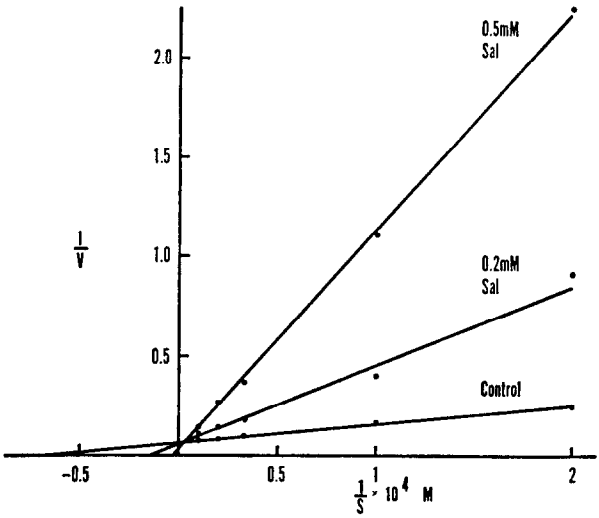

FIG. 5. Lineweaver–Burk plot illustrating the competitive inhibition of rat brain monoamine oxidase activity by salsolinol (SAL) (0·2 and 0·5 mM). K_i = 0·14 mM. Assays were performed as described in the Methods section. Serotonin concentrations ranged from 0·05 to 1·0 mM.

Gas chromatographic characteristics of methylated reaction products. Combined gas chromatographic radioactivity analysis revealed only one peak with carbon-14 activity when salsolinol was used as the substrate for COMT (Table 2). The retention time (6·8 min) of the methylated salsolinol product was identical to the retention time obtained with the 6-methoxy-7-hydroxy- and 6-hydroxy-7-methoxy-derivatives of salsolinol that are available as reference compounds. The monomethylated salsolinol derivatives were well separated from salsolinol and the dimethoxy derivative. Therefore, the metabolite(s) resulting from salsolinol incubation with COMT is one or both of the monomethylated derivatives.

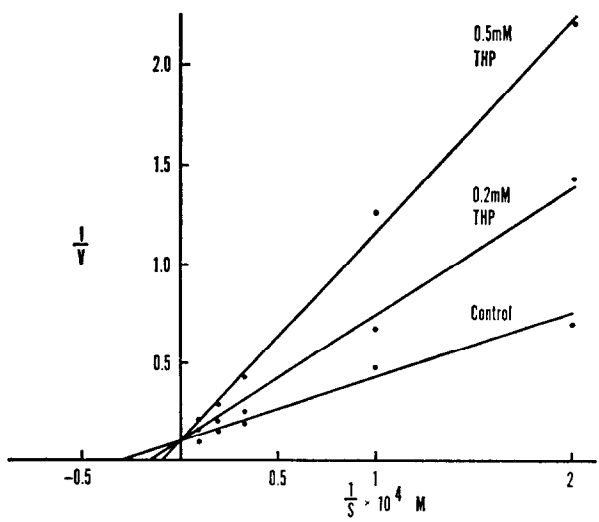

FIG. 6. Lineweaver–Burk plot of the competitive inhibition of rat brain monoamine oxidase activity by tetrahydropapaveroline (THP) (0·2 and 0·5 mM). K_i = 0·20 mM. Assays were performed as described in the Methods section. Serotonin concentrations ranged between 0·05 to 1·0 mM.

TABLE 2. GAS CHROMATOGRAPHIC CHARACTERISTICS OF TETRAHYDROISOQUINOLINE ALKALOIDS*

Compounds†	R_t	MU
O-methylated reaction product(s) of salsolinol	6·6	17·9
Salsolidine [1,2,3,4-tetrahydro-6,7-dimethoxy-1-methylisoquinoline]	5·8	17·4
Salsoline [1,2,3,4-tetrahydro-6-hydroxy-7-methoxy-1-methylisoquinoline]	6·6	17·9
1,2,3,4-Tetrahydro-6-methoxy-7-hydroxy-1-methylisoquinoline	6·6	17·9
Salsolinol [1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline]	7·8	18·5
O-methylated reaction products of tetrahydropapaveroline	8·5	27·9
	10·8	28·7
Reticuline [1,2,3,4-tetrahydro-6-methoxy-7-hydroxy-2-methyl-1-(4′-methoxy-3′-hydroxybenzyl)-isoquinoline]	7·4	27·3
Orientaline [1,2,3,4-tetrahydro-6-methoxy-7-hydroxy-2-methyl-1-(3′-methoxy-4′-hydroxybenzyl)-isoquinoline]	7·5	27·4
Norreticuline [1,2,3,4-tetrahydro-6-methoxy-7-hydroxy-1-(3′-hydroxy-4′-methoxybenzyl)-isoquinoline]	7·7	27·4
Laudanosoline [1,2,3,4-tetrahydro-6,7-dihydroxy-2-methyl-1-(3′,4′-dihydroxybenzyl)-isoquinoline]	9·5	28·3
Nororientaline [1,2,3,4-tetrahydro-6-methoxy-7-hydroxy-1-(3′-methoxy-4′-hydroxybenzyl)-isoquinoline]	9·7	28·4
Tetrahydropapaveroline [1,2,3,4-tetrahydro-6,7-dihydroxy-1-(3′,4′-dihydroxybenzyl)-isoquinoline]	12·2	29·0

* All compounds were analyzed isothermally as silyl derivatives. Gas chromatograph conditions were: 6 ft × 4 mm (i.d.) U-shaped glass column packed with 3% OV-1 on Gas Chrom Q, 100–120 mesh; detector temperature 290°; nitrogen flow rate, 60 ml/min; column temperature 190° and 260° for the 1-methyl- and 1-benzyltetrahydroisoquinoline alkaloids respectively. R_t = retention time (min); MU = methylene unit values.

† See Fig. 1 for basic structures.

Two radioactive products with retention times of 8·5 and 10·8 min were obtained on incubation of THP with COMT. These retention times did not correspond with those obtained with several methylated derivatives of THP that were available as reference standards—viz. reticuline, 7·4; orientaline, 7·5; norreticuline, 7·7; laudanosoline, 9·5 and nororientaline, 9·7 min (Table 2). The actual identification of THP's reaction products, necessitating the synthesis of each possible methylated derivative of that alkaloid, is currently under investigation.

DISCUSSION

The close structural similarity between catecholamine-derived tetrahydroisoquinoline alkaloids, such as salsolinol and THP, and the catecholamine neurotransmitters suggests that these alkaloids may be uniquely qualified to modify adrenergic function. Many of the recognized pharmacological actions of tetrahydroisoquinoline alkaloids are possibly reflective of augmented synaptic levels of catecholamine neurotransmitters.

The two major mechanisms in the termination of catecholamine-neurotransmitter action are reuptake by sympathetic neurons and enzymatic attack by COMT or MAO. Hence drugs interfering with either mechanism generally prolong or augment the activity of endogenous or exogenously administered catecholamines. Furthermore, direct adrenergic receptor stimulation by certain drugs mimics the response generated by catecholamine neurotransmitters.

The tetrahydroisoquinoline alkaloid derivatives of catecholamines have now been shown to alter both the uptake and storage,[22] as well as the enzymatic inactivation mechanisms for the catecholamines. Additionally, the complex benzyltetrahydroisoquinoline alkaloid derivative of dopamine, THP, acts as a direct β-sympathomimetic agent.[10]

As with the precursor neuroamines, the 1-methyltetrahydroisoquinoline alkaloids formed by the condensation of dopamine or norepinephrine with acetaldehyde are taken up, concentrated and stored in synaptosomes isolated from rat brains.[22] Additionally, these alkaloids interfere with the uptake of catecholamines and can also release existing stores of them.[22] Furthermore, the sympathetic nerves of the rat iris were observed to accumulate and store the alkaloid formed by the condensation of dopamine with formaldehyde, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline.[23]

COMT is the enzyme primarily responsible for the catabolism of catecholamines released at the sympathetic nerve endings. COMT catalyzes the transfer of the *S*-adenosyl-L-methionine methyl group to a phenolic group of substituted catechols and polyphenols, whether of natural or synthetic origin. Derived from the direct condensation of the deamination product of dopamine with the parent amine, THP is the requisite progenitor in the biosynthesis of many classes of even more complex alkaloids in plants—papaverine, tetrahydroberberine, aporphine and morphine, as examples.[24,25] The enzyme systems in plants that mediate THP biotransformations to these diverse alkaloid classes are as yet unidentified. But it is known that these biosynthetic mechanisms involve specifically directed *O*-methylation and oxidative phenol-coupling steps. As with mammalian systems, *S*-adenosyl-L-methionine in plants serves as the methyl donor for these methylation reactions. Additionally, desmethylpapaverine (papaveroline), the completely aromatized derivative of THP, markedly potentiates the physiological effects of catecholamines.[26] Burba and Murnaghan[26] have suggested that since this alkaloid strongly suppresses the rate of COMT-catalyzed methylation of norepinephrine *in vitro*, the potentiation of epinephrine responses is due to COMT inhibition. Furthermore, the bicyclic catechols, 6,7-dihydroxy- and 6,7-dihydroxy-*N*-methyl-1,2,3,4-tetrahydroisoquinoline, compounds closely related to salsolinol, have been shown to be substrates for COMT.[27] Thus, precedent for the possibility that the tetrahydroisoquinoline alkaloids derived from catecholamines and retaining vicinal hydroxyl groups are substrates for mammalian COMT certainly is not lacking.

The present documentation that salsolinol and THP are mammalian COMT substrates *in vitro* as well as competitive inhibitors of catecholamine *O*-methylation demonstrates another form of potential interaction between these alkaloids and catecholamines. The complex benzyltetrahydroisoquinoline alkaloid, THP, is of particular interest in this regard since it exceeds the simple 1-methyltetrahydroisoquinoline alkaloid, salsolinol, both in competitive inhibition of COMT and in evoking β-sympathomimetic responses.

An earlier investigation[28] revealed modest MAO inhibitory properties of salsolinol. At a concentration of 2·0 mM, the 1-methyltetrahydroisoquinoline alkaloid derivative of dopamine—salsolinol—caused approximately 50 per cent inhibition of dopamine deamination by rat brain stem homogenates.[28] The apparent K_i of salsolinol for serotonin deamination by rat brain stem MAO was given as 0·35 mM.[29] In the present studies, the simple alkaloid salsolinol and the complex alkaloid THP proved

to be equally potent competitive inhibitors of rat brain MAO with inhibitor constants of 0·14 and 0·20 mM respectively.

Whether the observed modest competitive inhibition of MAO by salsolinol and THP is of significance in the pharmacological actions of these alkaloids is debatable, since the K_i values observed were considerably greater than the K_i of $5{\cdot}8 \times 10^{-5}$ mM reported by Hellerman and Erwin[30] for tranylcypromine, a potent substrate competitive inhibitor of mitochondrial MAO. However, K_i values for salsolinol and THP are comparable to the I_{50} (0·7 mM) reported by Maass and Nimmo[31] for iproniazid as an inhibitor of serotonin oxidation by rat brain MAO. Thus one cannot entirely ignore the possibility that some of salsolinol's and THP's pharmacological effects might be a consequence of MAO inhibition.

The tetrahydroisoquinoline alkaloids related to the catecholamines can now be added to the list of inhibitors of the major enzymatic routes for the disposition of the catecholamines. These inhibitors are known to augment or prolong the physiological responses induced by the catecholamines. *In situ* production of these aberrant neuroamine alkaloid metabolites would place them in highly vulnerable locales of the peripheral and central adrenergic systems. At such sites these metabolites might manifest some of their diverse pharmacological actions in the modification of catecholamine function by: (1) acting as potential false transmitters, (2) competing with the catecholamines for binding sites and (3) interfering with the enzymatic systems responsible for the metabolic disposition of the catecholamines.

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