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TETRAHYDROISOQUINOLINES DERIVED FROM CATECHOLAMINES OR DOPA: EFFECTS ON BRAIN TYROSINE HYDROXYLASE ACTIVITY*

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Abstract—Four different catecholic tetrahydroisoquinolines, cyclic condensation derivatives of L-DOPA, dopamine or noradrenaline and selected aldehydes, were examined for inhibition of rat brain tyrosine hydroxylase activity. Salsolinol, a condensate of dopamine and acetaldehyde which is implicated in the biological effects of alcohol, inhibited tyrosine hydroxylase *in vitro* with a K_i of $\sim 1.4 \times 10^{-5}$ M; inhibition was competitive with pteridine cofactor. The (−)isomer of salsolinol was significantly more effective than (−)noradrenaline. Tetrahydroisoquinolines with *in vitro* inhibiting potency equivalent to (−)noradrenaline were (*cis*)-3-carboxy-salsolinol, an L-DOPA/acetaldehyde derivative, and 4,6,7-trihydroxy-tetrahydroisoquinoline, a product of noradrenaline and formaldehyde. At 10^{-4} M, however, neither salsolinol nor 3-carboxy-salsolinol had any *in vitro* effect on rat liver DOPA decarboxylase activity. Tetrahydropapaveroline, a 1-benzyl derivative of dopamine also theorized to be involved in alcoholism, was markedly and significantly less effective than the other condensation products, since it inhibited tyrosine hydroxylase at relatively high ($\sim 10^{-3}$ M) concentrations only. Labeling of catecholamines in brain and heart by [^{3}H]-tyrosine was not changed 5 hr after peripherally administered salsolinol, indicating that this tetrahydroisoquinoline did not alter tyrosine hydroxylase *in vivo* under these conditions. However, it is possible that tyrosine hydroxylase is affected at other doses or times, and that the blood–brain barrier is responsible for the lack of central effects. Inhibition of catecholamine biosynthesis remains a viable possibility if catechol tetrahydroisoquinolines are administered centrally or formed in neurons from condensations of catecholamines and aldehydes.

Catechol tetrahydroisoquinoline analogs of the catecholamines (CA) or possibly 3,4-dihydroxyphenylalanine (DOPA) have been proposed as aberrant metabolites forming during certain neuropathological states. Particular attention has focused on tetrahydroisoquinolines from potential CA condensations with acetaldehyde during alcohol metabolism [1, 2] and from L-DOPA with endogenous aldehydes during therapy for Parkinson's disease [3, 4].

Since the catechol tetrahydroisoquinolines or their *in vivo* metabolic products may have varying neuronal effects, studies of their effects on CA storage, uptake and release processes, on CA receptors, and on the CA catabolic enzymes, monoamine oxidase (MAO) and catechol-*O*-methyl transferase, have been carried out [2]. It is well-established that catechol compounds are often good inhibitors of tyrosine hydroxylase, the rate-limiting enzyme in the CA biosynthetic pathway [5, 6]. Endogenous noradrenaline (NA) and perhaps dopamine (DA) may have a physiological function in this regard as negative feedback inhibitors [7]. Alternatively, some catechols are relatively inactive as tyrosine hydroxylase inhibitors [8].

In the one available observation of the effect of a catechol tetrahydroisoquinoline on catecholamine biosynthesis, Patrick and Barchas [9] reported that salsolinol (stereochemistry undefined) inhibited the incorporation of radioactive tyrosine into synaptosomal dopamine. In the present study, our objective was to determine the extent and nature of tyrosine hydroxylase inhibition by various tetrahydroisoquinolines (Fig. 1). Emphasis was placed on salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), the product of acetaldehyde and DA which has been detected in brains of rats during ethanol metabolism with high blood acetaldehyde levels [10], and in the urine of DOPA-treated Parkinson's disease patients [4]. The effect of intraperitoneally (i.p.)

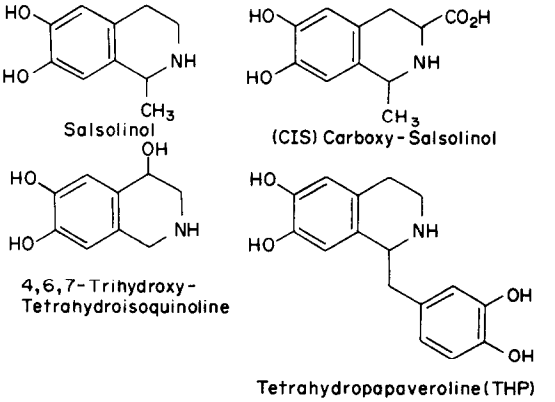

Fig. 1. Tetrahydroisoquinolines related to dopamine, noradrenaline and L-DOPA used in this study.

* A portion of this study was reported at the Second Int. Symp. on Alcohol and Aldehyde Metab. Systems, Philadelphia PA, October 1976.

administered salsolinol on the accumulation of [^{3}H]-catecholamines in rat brain areas and rat heart after intravenous (i.v.) [^{3}H]tyrosine was examined also.

MATERIALS AND METHODS

Materials. In Fig. 1, salsolinol (HCl salt), (±)4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (HCl salt), and 1*S*,3*S*(*cis*)-3-carboxy-salsolinol (derived from L-DOPA according to Ref. 11) were synthesized in this laboratory and were > 98 per cent pure by gas–liquid chromatography [12]. The *S*(−) and *R*(+) stereoisomers of salsolinol, as well as the L-DOPA decarboxylase inhibitor, RO4-4602, were obtained from the Hoffmann-LaRoche Co., Nutley, NJ. (±)Tetrahydropapaveroline [THP: 1-(3′,4′-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline HBr] was obtained from the Wellcome Research Laboratories, Beckenham, U.K.

Tyrosine hydroxylase assays. Whole brains, without cerebella, from adult male Sprague–Dawley rats were homogenized (10%, w/v) in ice-cold 50 nM Tris acetate buffer (pH 6) containing 0.2% Triton X-100. Generally, five to seven rat brains were pooled for a group of assays. Portions (400 μl) of the supernatant solution obtained from 15-min centrifugation (15,000 *g*) of the brain homogenate were preincubated for 5 min at 37° with 1 nmole pteridine synthetic cofactor $DMPH_4$ (CalBiochem, La Jolla, CA), 1 nmole $Fe(NH_4)(SO_4)_2 \cdot 6H_2O$, 20 nmoles mercaptoethanol, and the tetrahydroisoquinoline or CA in 0.95 ml of 0.2 N NaOAc (pH 6) buffer. L-Tyrosine (50 nmoles), containing 6×10^5 cpm [3,5-^{3}H]L-tyrosine (41–54 Ci/m-mole: New England Nuclear, Boston, MA) which had been prepurified on AG-50 ion exchange column, was added in 0.5 ml of distilled H_2O to initiate the reaction. The reaction was terminated after 30 min with 0.1 ml of 25% trichloroacetic acid, and was centrifuged for 10 min at 2500 rev/min (clinical centrifuge). The supernatant solution and 1 ml H_2O were passed over AG-50 columns, and the effluents were collected and counted in a 15-ml scintillation mixture (toluene: 0.4% PPO: 20% Bio-Solv BBS-111).* Recovery of [^{3}H]H_2O from the columns varied from 73 to 85 per cent. A reagent blank measured non-enzymatic release of [^{3}H]H_2O.

[^{3}H]*catecholamine synthesis* in vivo *from* [^{3}H]-*tyrosine.* Unanesthetized male Sprague–Dawley rats (300–400 g) were treated with salsolinol HCl (50 mg/kg, i.p) or saline, and after 4 hr 40 min, were injected via the tail vein with 0.5 ml of prepurified [3,5-^{3}H]-tyrosine (~50 Ci/m-mole: 50 μCi/rat). Twenty min later, rats were stunned and decapitated. Brains were removed and rinsed briefly in ice-cold saline. The striatal and hypothalamic areas were dissected, weighed and frozen on dry ice. Hearts were blotted free of blood, weighed and frozen. The tissues were homogenized in cold 0.5 N perchloric acid (3 ml, striata or hypothalami: 6 ml, rest of brain or heart) containing 5 mM sodium metabisulfite. Samples were centrifuged for 30 min (4°) at 2500 rev/min, and the supernatant fluid was decanted. The pellet was re-homogenized briefly in 0.5 vol. of fresh cold perchloric acid solution and recentrifuged as before. After removal of aliquots for scintillation counting, the combined supernatant solutions were adjusted in scintillation vials to pH 8.2–8.5 with 1 N NaOH. Purified aluminium oxide (Al_2O_3: 100 mg) was added, and the vials were capped and shaken for 12 min at room temperature. The supernatant fluids were discarded, and the Al_2O_3 was washed (3 × 5 ml distilled H_2O) and dried under vacuum at 35° for 10 min. The 2.0 ml of 0.1 N HCl was added to each vial, and they were capped and shaken. After 10 min the acid was decanted through a small glass wool-packed column into a 15-ml scintillation mixture and counted in a Beckman LS-250 liquid scintillation system. Overall recoveries ranged from 58 to 75 per cent. Counting efficiencies were estimated by the internal standard method.

* PPO = 2,5-diphenyloxazole.

RESULTS

Since assays of tyrosine hydroxylase activities were linear for at least 30 min (Fig. 2), reactions were terminated at this time. Results of tyrosine hydroxylase assays at subsaturating substrate and $DMPH_4$ concentrations and with varying concentrations of (−) NA, THP, 4,6,7-trihydroxy-tetrahydroisoquinoline, or the salsolinol stereoisomers, are graphed in Fig. 3. As an inhibitor of tyrosine hydroxylase activity, *S*(−)salsolinol was significantly more effective than either (−)NA or the tetrahydroisoquinolines, THP and 4,6,7-trihydroxy-tetrahydroisoquinoline, at 5×10^{-4} to 5×10^{-5} M. The *laevo*(−) isomer also showed slightly more inhibition than *R*(+) salsolinol, but this difference was not statistically significant.

4,6,7-Trihydroxy-tetrahydroisoquinoline was approximately equal to its CA precursor, (−)NA, as an inhibitor of tyrosine hydroxylase. However, both the 4-hydroxy derivative and (−)NA were significantly better inhibitors of tyrosine hydroxylase than THP at two of the concentrations examined.

Table 1 presents the results of comparative assays of the activities of tyrosine hydroxylase and DOPA decarboxylase in the presence of 10^{-4} M (racemic)

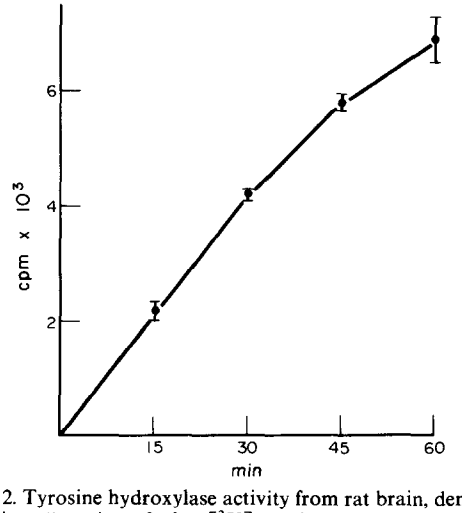

Fig. 2. Tyrosine hydroxylase activity from rat brain, demonstrating linearity of the [^{3}H]tyrosine assay with time. Values are means (± S. D.) of triplicate determinations after subtraction of blanks. Blanks were in the range of 750–1200 cpm.

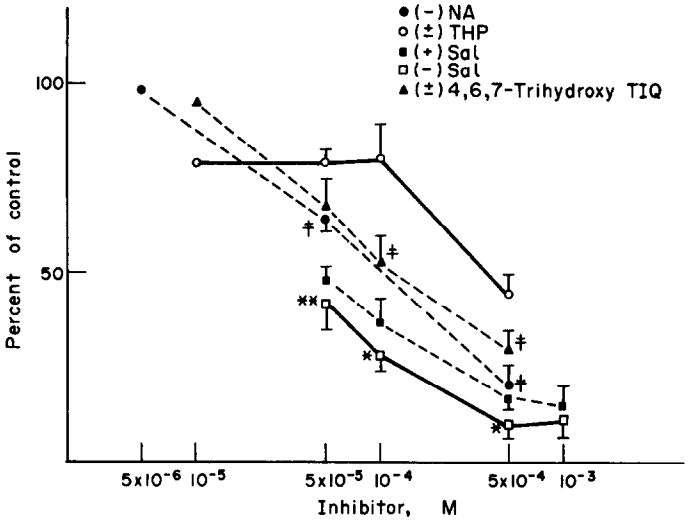

Fig. 3. Effect of *S*(−)salsolinol, *R*(+)salsolinol, 4,6,7-trihydroxytetrahydroisoquinoline (TIQ), tetrahydropapaveroline (THP), and (−) noradrenaline (NA) on tyrosine hydroxylase activity from rat brain. Assays were performed as described in the text. Shown are the means (±S. D.) of three to four determinations. Control values (less blanks) were 3700–4500 cpm. A single asterisk (*) indicates significantly different ($P < 0.05$) from 4,6,7-trihydroxy-TIQ and THP at this concentration. A double asterisk (**) indicates significantly different ($P < 0.05$) from (−)NA, 4,6,7-trihydroxy-TIQ or THP at this concentration. A double dagger (‡) indicates significantly different ($P < 0.05$) from THP at this concentration.

(±)salsolinol, (*cis*)-3-carboxy-salsolinol (DOPA/acetaldehyde condensation product), L-DOPA, or the decarboxylase inhibitor RO4-4602 (benserazide). Racemic salsolinol displayed the greatest degree of inhibition of tyrosine hydroxylase, and was ineffective as a DOPA decarboxylase inhibitor. (*cis*)-3-Carboxy-salxolinol was equivalent to its precursor (L-DOPA) as an inhibitor of tyrosine hydroxylase activity. However, neither the amino acid-derived tetrahydroisoquinoline nor (±)salsolinol was an inhibitor of DOPA decarboxylase from rat liver under conditions at which RO4-4602 inhibited decarboxylase activity > 95 per cent.

Figure 4, a reciprocal plot, shows the change in tyrosine hydroxylase activity with respect to the cofactor ($DMPH_4$) at three different salsolinol concentrations. Lines were generated by the method of least squares. Inhibition by (±)salsolinol at 1×10^{-5} M and 2.5×10^{-5} M appeared purely competitive with the pteridine cofactor, since V_{max} remained unchanged as K_m (apparent) increased. (±)Salsolinol did not change the K_m for L-tyrosine substrate, however. The slopes (K_m/V_{max}), K_i values and standard deviations from Fig. 4 are shown in Table 2. The mean K_i calculated from the slopes was $1.38 \pm 0.35 \times 10^{-5}$ M.

Attempts were made to determine if salsolinol, given peripherally, altered the activity of brain tyrosine

Table 1. Comparison of the *in vitro* inhibition of tyrosine hydroxylase and DOPA decarboxylase by (±) salsolinol, (*cis*)-3-carboxy-salsolinol, L-DOPA, or RO4-4602*

Compound	Per cent inhibition at 10^{-4} M Tyrosine hydroxylase	DOPA decarboxylase
(±)Salsolinol	62	< 3
(*cis*)-3-Carboxy-salsolinol	48	< 5
L-DOPA	40	5
RO4-4602	†	96

* Rat brain tyrosine hydroxylase assays were carried out as described in Materials and Methods. Rat liver DOPA decarboxylase assays, carried out by Dr Allen Frankfater, employed the method of Christensen *et al.* [13] measuring $[^{14}C]CO_2$ evolution from the $[^{14}C]$DOPA substrate. RO4-4602 is a widely used DOPA decarboxylase inhibitor. Results are averages of triplicate determinations. Control values (less blanks) were in the range of 3700–4500 cpm.

† Not determined.

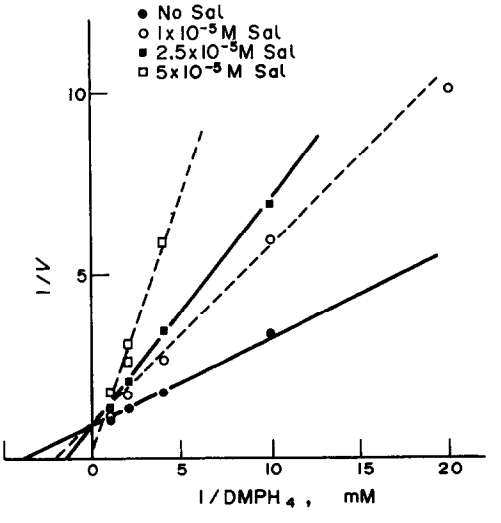

Fig. 4. Lineweaver–Bürk plot of velocity vs $DMPH_4$ concentration at varying concentrations of (±)salsolinol (Sal).

Table 2. Kinetic parameters for the inhibition of tyrosine hydroxylase by (±)salsolinol

Sal × 10^{-5} M	K_{app}/V_{max}(sec)	K_i (calc) × 10^{-5} M*
0	0.261 ± 0.009	
1.0	0.476 ± 0.017	1.22 ± 0.06
2.5	0.627 ± 0.023	1.79 ± 0.10
5.0	1.407 ± 0.080	1.14 ± 0.08

* Mean K_i calculated from the slopes was 1.38 ± 0.35 × 10^{-5} M.

Table 3. Effect of salsolinol (i.p.) on the accumulation of [^{3}H]catechols after intravenous [^{3}H]tyrosine in rats*

Tissue or brain region	Per cent tritium as catechols Salsolinol	Saline	P
Striatum	3.62 ± 0.20	3.42 ± 0.34	NS
Hypothalamus	6.36 ± 1.71	5.56 ± 1.17	NS
Rest of brain	0.38 ± 0.03	0.40 ± 0.04	NS
Heart	0.28 ± 0.02	0.25 ± 0.05	NS

* Male Sprague–Dawley rats (300–400 g) were given salsolinol (50 mg/kg) or isotonic saline i.p., and 4 hr 40 min later, [3,5-^3H]tyrosine via tail vein. They were stunned and decapitated 20 min after [^{3}H]tyrosine. [^{3}H]catechols were isolated as described in Materials and Methods. Results are expressed as means ± S. E. M. NS = not significant. Six rats per group were used.

hydroxylase *in vivo*. Accumulation of labeled catechols (NA, DA) in rat brain areas and in heart was determined 20 min after i.v. [^{3}H]tyrosine administration to salsolinol- or saline-pretreated rats. As shown in Table 3, there were no significant differences between catechol labeling in these tissues. An alternative approach was to assay (using conditions identical to the *in vitro* inhibition studies) the activity of tyrosine hydroxylase solubilized from striata of rats treated 2 hr previously with saline or salsolinol (50 mg/kg, i.p.). The results (C. D. Weiner and M. A. Collins, unpublished data) supported the [^{3}H]catechol labeling experimental findings, since there was no difference between salsolinol- and saline-treated rats with respect to brain tyrosine hydroxylase activity.

DISCUSSION

In this report, we consider the possibility that tetrahydroisoquinolines derived from CA or L-DOPA can affect CA biosynthesis, particularly via direct inhibition of tyrosine hydroxylase. Encouraged initially by a report [9] showing inhibition of DA formation from [^{14}C]tyrosine by salsolinol in rat synaptosomal preparations, we have extended this finding to show that a range of catechol tetrahydroisoquinolines derived potentially from condensations of simple aldehydes (formaldehyde, acetaldehyde) with CA, including the stereoisomers of salsolinol, are effective as inhibitors of soluble tyrosine hydroxylase activity from rat brain. Racemic salsolinol was found to inhibit the rate-controlling enzyme competitively, with a K_i (calc) of 1.38 × 10^{-5} M. Its *S*(−) isomer was a more potent inhibitor than (−)NA, an accepted physiological feedback regulator. The failure of 10^{-4} M salsolinol to alter DOPA decarboxylase activity (Table 1) indicates that the inhibition by salsolinol of [^{14}C]dopamine synthesis from [^{14}C]tyrosine in synaptosomes [9] was due principally to its effect on tyrosine hydroxylase. At higher levels ($\sim 10^{-2}$ M), salsolinol is reported to have an inhibitory effect *in vitro* on liver decarboxylase activity [14].

Tyrosine hydroxylase inhibition was also demonstrated by two other "simple" (i.e. hydrogen or methyl group in the 1-position) catechol tetrahydroisoquinolines. One of these, (*cis*)-3-carboxy-salsolinol, has been observed, along with salsolinol, to have analgesic effects in mice [15]. It is possible that inhibition of catecholamine biosynthesis was involved in this behavioural effect of carboxy-salsolinol. A 4-hydroxylated tetrahydroisoquinoline related to adrenaline was found to deplete NA in guinea pig heart and hypothalamus [16]. Tyrosine hydroxylase inhibition could reasonably underlie this result since we have found that the 4-hydroxylated condensation product of NA and formaldehyde was as effective an inhibitor as NA.

It is interesting to note that the tetrahydroisoquinoline condensation product with relatively negligible *in vitro* effects on tyrosine hydroxylase was the 1-benzyl derivative, THP. This tetrahydroisoquinoline, a morphine precursor in plants, has drawn attention since 1970 when it was proposed as a molecular bridge between alcohol and opiate addictions [17]. While its effects on brain CA are not known, THP, given i.p., does cause an early increase in the DA metabolite, homovanillic acid, which is consistent with CA release rather than with inhibition of CA synthesis [18]. Small quantities of THP were excreted by Parkinsonian patients taking L-DOPA [4], but using a high performance liquid chromatography procedure, we were unable to detect any THP in urines from alcoholics during detoxification.*

At a peripheral dose known to cause moderate decreases in brain DA and heart NA concentrations [19], salsolinol had no effect on *in vivo* tyrosine hydroxylase activity measured by [^{3}H]tyrosine metabolism (Table 3). It is possible that inhibition of CA biosynthesis would have been apparent at shorter times after salsolinol administration, or if a more sensitive indicator of activity, such as DOPA accumulation after inhibition of central decarboxylases [20], were employed. Additionally, the certainty of a significant blood/brain barrier to salsolinol (M. A. Collins, unpublished results) rules against large alterations in central catecholaminergic systems by peripherally administered catechol tetrahydroisoquinolines. Salsolinol and various other isoquinolines are currently being re-examined using intraventricular techniques and the DOPA accumulation assay.

If CA-derived tetrahydroisoquinolines should form in sufficient quantities within neurons during alcohol intoxication, it would be difficult to separate their possible effects on CA regulatory enzymes from the effects of ethanol, acetaldehyde, other metabolic intermediates whose levels change secondarily, or released CA. Furthermore, there is no consistent

* M. Collins and W. P. Nijm, manuscript in preparation.

experimental picture of the effects of intoxication on CA biosynthesis and turnover. The confusion existing in this area was reviewed recently by Tabakoff [21]. Differences and variations in the route and duration of ethanol administration, dosages, subsequent blood ethanol and acetaldehyde levels, animal species and strains, and analytical methods have resulted in conflicting results.

Nevertheless, there are several studies on DA biosynthesis and chronic ethanol which are relevant. One such study, not mentioned in the preceding review, was reported by Friedhoff and Miller [22]. With the [^{3}H]tyrosine *in vitro* assay, the authors found that caudatal tyrosine hydroxylase activity in rats exposed to ethanol/metrecal diets for 100 days was reduced significantly (21.5 per cent) compared to pair-fed sucrose/metrecal controls. It is possible that a CA-condensation product stored in nerves was responsible in part for this inhibition. However, it is also likely that protein synthesis and, particularly, tyrosine hydroxylase levels were depressed by the long exposure to alcohol and its metabolites.

In another study of shorter duration, Hunt and Majchrowicz [23] found that dependence induced by ethanol intubation for 4 days resulted in a decrease in the rate of disappearance of brain DA in α-methyltyrosine-treated rats, indicating reduced DA turnover during intoxication. On the other hand, in the same alcoholic model, Karoum *et al.* [24] reported that, during the intoxication phase, whole brain concentrations of the principal DA metabolites, homovanillic acid (HVA) and dihydroxylacetic acid (DOPAC), were increased 30–45 per cent. These investigators believed that these metabolite changes reflected an ethanol-induced increase in DA turnover, rather than a decrease in brain efflux of HVA and DOPAC, or increased DA release. Decreased efflux was ruled out because the rates of disappearance of brain HVA and DOPAC appeared identical in acute ethanol–pargyline- or control pargyline-treated rats. However, extrapolating from experiments involving acute ethanol and pargyline, an MAO inhibitor which also inhibits aldehyde dehydrogenase and elevates brain acetaldehyde levels 10- to 20-fold [25], to experiments with chronic ethanol only, is highly suspect.

In these two studies with the intubation model, the withdrawal phase was characterized by decreased DA turnover rates, while turnover rates for NA remained elevated. It is tempting to explain this divergent result by the selective presence of inhibitory CA-derived condensation products in dopaminergic neurons. At present there is only limited evidence for detectable concentrations of tetrahydroisoquinolines in chronic alcohol-treated animals. O'Neill and Rahwan [26] were unable to detect salsolinol in the brains of mice exposed chronically to ethanol, but Hamilton and Blum [27] have found gas chromatographic evidence for substantial amounts of the 6-*O*-methylated salsolinol product (isosalsoline) in brain samples of mice treated in the same manner.

While evidence for an effect of administered tetrahydroisoquinolines on central CA turnover is still lacking, studies are in progress with the carboxylated isomers (carboxy-salsolinol) which are predicted to cross the blood–brain barrier. In addition, the interaction of tetrahydroisoquinolines with synaptic receptors as a mechanism of action on CA biosynthesis must be considered, although available evidence indicates that simple DA-related tetrahydroisoquinolines are, at best, weak receptor agonists [28].

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