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Half-lives of salsolinol and tetrahydropapaveroline hydrobromide following intracerebroventricular injection

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Although interest in the physiological action of tetrahydroisoquinolines (TIQs) was sparked by the hypothesis that these compounds might be formed following the ingestion of alcohol [1, 2], progress in testing this theory stalled due to failure to detect the formation of these amine–aldehyde condensation products after the administration of alcohol [3]. However, behavioral tests in rodents support the notion that the presence of TIQs may underlie some of the actions of alcohol. TIQs can exacerbate the effects of ethanol on sleeping time [4] or enhance the severity of withdrawal from ethanol [5]. Infusion of various TIQs into a cerebral ventricle of the rat induces the animal to ingest voluntarily large quantities of alcohol [6–8]. In the infusion experiments, the minute quantities of TIQ employed to produce behavioral alterations suggest that only exceedingly small amounts of these compounds would need to be formed following alcohol ingestion in order to influence behavior.

Since the importance of the involvement of TIQs in the action of alcohol is dependent on demonstrating that they are formed following the ingestion of alcohol alone, estimating the brain levels achieved during chronic infusion experiments would indicate the level of sensitivity required for an assay to detect levels of these compounds capable of influencing behavior. Until now, there has been no indication of the minimum level of sensitivity required. In order to make estimates of the level of assay sensitivity required, the half-lives of salsolinol and tetrahydropapaveroline hydrobromide (THP) are needed. This paper describes the determination of these values.

Female rats of the Long–Evans strain, weighing 200–300 g, were anesthetized with an intraperitoneal injection of 30 mg/kg of sodium pentobarbital. The animal was then placed in a stereotaxic instrument in the DeGroot orientation. The scalp was incised and a burr hole was drilled in the skull at AP 5.8, 1.5 mm lateral to the midline. After lowering a 23 gauge injection needle 3.0 mm into the brain so that the tip rested in the ventricle, 1.0 μg salsolinol hydrobromide or 10.0 μg THP in 10.0 μl of artificial cerbrospinal fluid [9] were injected. The animals were killed by cervical dislocation at intervals after the injection. The whole brain was rapidly removed, frozen in liquid nitrogen, and weighed. The brains were kept frozen at − 70° until assayed later the same day.

Both salsolinol and THP have been shown to be metabolized *in vitro* via *O*-methylation [10]. Therefore, in a second experiment, 250 mg/kg of pyrogallol, an inhibitor of catechol-*o*-methyltransferase, was administered intraperitoneally 30 min prior to the intraventricular injection of the TIQ.

Brains were homogenized at room temperature in 6 ml of a solution of 0.5 M HCl, 0.1 M $HClO_4$, and 1% sodium metabisulfite with 100 ng of 3,4-dihydroxybenzylamine added to each sample as an internal standard. The homogenate was centrifuged at 2000 *g* for 10 min at 0°. The supernatant fraction was transferred to a 10 ml beaker containing 100 μl each of 5% sodium metabisulfate and 10% EDTA disodium salt. The pH was then adjusted to 8.5 with NaOH. The sample was immediately placed in a conical vial containing 80 mg of alumina which had been washed previously with a 1:1 mixture of $CHCl_3$ and CH_3OH. The suspension was shaken for 12 min at room temperature. The liquid was then aspirated and the alumina was washed three times with distilled water. After the final aspiration, 400 μl of 1 N acetic acid were added to the alumina and shaken for 10 min. This eluate was then assayed using high-pressure liquid chromatography with an electrochemical detector [11]. A pellicular Vydac SCX stationary phase was dry packed in a 50 cm × 2 mm i.d. glass column. The mobile phase consisted of a mixture of 380 ml of 0.1 M citric acid, 320 ml of 0.2 M Na_2HPO_4, and 200 ml of distilled deionized water with 1 drop of toluene added to each liter to prevent microbial growth. The detector potential was set at 0.7 V. The flow rate was 0.4 ml/min, generating a column pressure averaging 500–600 psi.

The amount of salsolinol recovered as a percentage of that injected at different time points is given in Fig. 1. The half-life of this compound in the brain was determined to be 12.5 min. As shown in Fig. 1, administration of 250

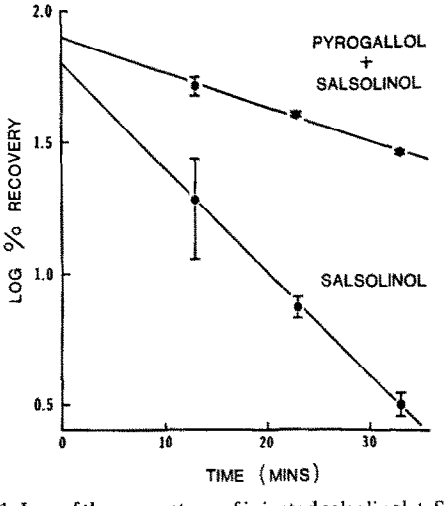

Fig. 1. Log of the percentage of injected salsolinol ± S.E.M. recovered at different times after the intraventricular injection of 1.0 μg salsolinol, with (N = 9) and without (N = 44) prior intraperitoneal administration of 250 mg/kg of pyrogallol.

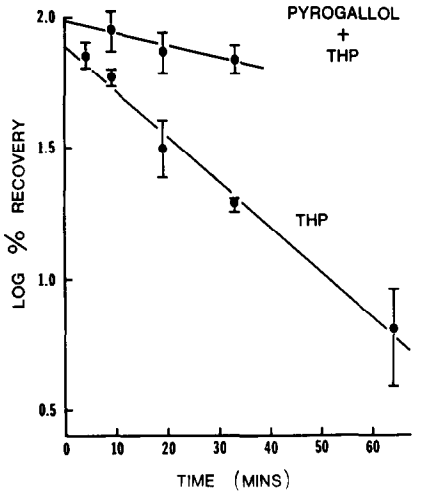

Fig. 2. Log of the percentage injected THP ± S.E.M. recovered at different times after the intraventricular injection of 10.0 μg THP, with (N = 8) and without (N = 18) prior intraperitoneal administration of 250 mg/kg of pyrogallol.

mg/kg i.p. of the catechol-*o*-methyltransferase inhibitor, pyrogallol [12], prior to the injection of salsolinol increased the half-life of this compound to 23.1 min. This supports the evidence already available that salsolinol serves as a good substrate for *O*-methylation [10].

In chronic infusion experiments, an infusion rate of 40 ng every half-hour, the lowest dose of salsolinol tested, caused an increase in alcohol consumption [8]. With a half-life of 12.5 min, it can be calculated from the equation

$$X^{oo} = \frac{X_0}{1 - e^{-k_0 td}}$$

(where X^{oo} is the amount of compound at $t = oo$, X_0 = the dose, k_0 = the disappearance rate constant and td is the dosing interval) that the peak amount steady state would be 49.0 ng per whole brain. Assays easily capable of detecting this amount of salsolinol or *O*-methyl salsolinol are available [13].

The amount of THP present in the brain at various intervals as a percentage of that given after injection is shown in Fig. 2. From the amount of THP recovered at different time points, a half-life of 17.3 min was calculated. The administration of 250 mg/kg of pyrogallol extended the half-life of THP to 69.3 min. This is a greater increase in $t_{1/2}$ than that observed for salsolinol and argues for the fact that *O*-methylation is a route of metabolism for this compound *in vivo* [10].

The lowest dose of THP tested in the chronic infusion experiments was 0.4 ng every half-hour [7]. With a half-life of 17.3 min, it is calculated that the maximum amount present at steady state would be 0.6 ng in the whole brain (0.3 ng/g). This is below the level of detection (2 ng/g) for the most sensitive assay which has yet been used to determine whether or not TIQs form after the ingestion of alcohol [11]. It is likely that even lower doses than those used in the original infusion experiments would be capable of altering behavior.

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Morphine-induced depression of the hepatic microsomal drug-metabolizing enzyme—effect on the lipid component

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The oxidation of drugs in the liver is mediated primarily by an enzyme complex consisting of the proteins, cytochrome P-450 and NADPH-cytochrome P-450 reductase, and the phospholipid, phosphatidylcholine [1–5]. Resolution and reconstitution of the enzyme system have revealed the role of each component in the overall catalysis which requires molecular oxygen and NADPH [1]. The hemeprotein, cytochrome P-450, is the site of substrate binding, oxygen activation and oxidation. NADPH-cytochrome P-450 reductase functions as an electron carrier, transferring reducing equivalents from NADPH to cytochrome P-450. The role of the phospholipid is to provide a suitable