Ethanol Effects on Brain Concentrations of Amitriptyline and the Relationship to Psychomotor Function

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Abstract. The effect of amitriptyline (AMI), ethanol (ETOH), and ETOH followed by AMI on both general activity (open field) and motor performance (two rotorod tasks) was tested in reference to a saline-injected control. The combination (ETOH plus AMI) produced greater impairment on all three tasks than did either drug alone. ETOH pretreatment also produced a 223% increase in the total tricyclic antidepressant (TCA) brain concentration. The decrement in motor performance was logarithmically related to total TCA brain concentrations in both animals treated with AMI alone and those pretreated with ETOH prior to AMI. The concentration which consistently produced behavioral impairment was similar to those previously reported to cause cognitive and electroencephalographic dysfunction in humans.

Key words: Amitriptyline – Open field – Ethanol – Rotorod – Psychomotor performance

Patients receiving tricyclic antidepressants (TCA) are routinely advised to avoid ethanol (ETOH). Concomitant ingestion of these drugs produces greater toxic effects than either agent alone. This phenomenon has been best studied with regard to impairment of psychomotor coordination and sedation and is a significant health problem, being the cause of serious accidents (Lander et al. 1969; Seppala 1977). The mechanism has been routinely attributed to the additive CNS depressant effects of the drugs. However, concomitant ingestion of TCA and alcohol also causes more serious cardiovascular (CV) toxicity, such as fatal arrhythmias (Preskorn and Irwin 1982). This latter observation cannot be explained solely on the basis of additive cardiotoxicity of the two drugs and suggests a possible pharmacokinetic interaction.

Patients receiving standard doses of TCA can experience both CNS and CV toxicity due to slow metabolism and the development of inappropriately high plasma and presumably tissue concentrations of the drugs. When plasma levels of amitriptyline (AMI) and its major metabolite nortriptyline exceed 450 ng/ml, patients are at high risk of development of drug-induced impairment in CNS function as witnessed by the development of a toxic-confusional state with associated electroencephalographic (EEG) disturbances (Preskorn and Simpson 1982; Preskorn et al. 1983). A principal determinant of the drug concentration a subject will achieve on a given dose of a TCA is liver function. These drugs normally undergo extensive first-pass metabolism prior to reaching plasma and tissue compartments of the body. For example, the same brain concentration of AMI can be achieved with an IV dose 50-times less than an IP or oral dose by avoiding first-pass liver metabolism (Glotzbach and Preskorn 1982). Since ETOH is well known to have both subacute and chronic effects on the ability of the liver to metabolize a wide variety of drugs, it might well alter the amount of AMI reaching the target tissue. Given this background, we decided to further examine the concentration-dependent nature of AMI-induced CNS toxicity in laboratory animals by assessing the relationship between brain concentrations of AMI and impairment in psychomotor coordination in animals treated with AMI alone and in animals treated with ETOH prior to receiving AMI.

Materials and Methods

Adult male Sprague-Dawley rats (mean weight 306 ± 5.7 g) that were reared in our laboratory were housed singly under 12-h light-dark cycle and provided food and water ad libitum.

Pretesting. All rats were initially tested in an automated open field for ambulatory activity for 10 min. The enclosed open field consisted of 36 15.24 cm squares (six by six). Each square was independently suspended and designed so that the weight of an animal placed on any portion of the square activated a switch (Hughes 1978). A cable connected the switches of the arena to a minicomputer. A program monitored the changes in the rat’s position, traced the path taken by the animal, and recorded the totals of inner and outer square crossings minute by minute.

Upon completion of the open field test, the rats were trained to stay on a 5 cm diameter rotorod rotated at 5 rpm. To keep from falling 1.25 m to the floor, a rodent had to walk and maintain balance on a knurled cylinder rotating at the preselected speed (Rotamax, Columbus Instruments, Columbus, OH, USA). This task of motor coordination has been found in many different situations to be sensitive to both drug dosage and toxicity. Increasing either the cylinder rpm or the diameter make remaining on the cylinder more difficult. Only rats that completed a 180-s trial without falling off were included in the study.

Testing. After 2 days, nine rats received 17.5 mg/kg IP AMI, 18 received 1 g/kg IP ETOH, and 19 received 1 g/kg IP ETOH followed in 2 min by 17.5 mg/kg AMI IP (AMI + ETOH).
Ten control rats received an equivalent volume injection of saline. Assignment to groups assured a nearly equivalent initial mean open field activity score for each group. At 15 min after the last injection, each rat was given a second 10-min exposure to the open field. Following the open field test, each was given three trials on the 5 cm diameter rotorod apparatus at 12 rpm, and then for an additional measure with increased difficulty, three trials on the 10 cm diameter rod at 12 rpm (a maximum of 90 s per trial). The total latencies for remaining on the 5 and 10 cm rod were recorded. Behavioral testing occurred during a period in which the maximal concentration of drug in the brain should have been obtained (Glotzbach and Preskorn 1982).

**Drug Assays.** At 45 min postinjection, the rats were decapitated and a trunk blood sample was taken. The brains from all rats given AMI were then rapidly removed, frozen in liquid nitrogen, and stored at −70°C. The procedure for determining AMI concentrations in the brain has been described previously (Preskorn and Glotzbach 1982) and is briefly described here.

A chromatograph (Waters Associates, Milford, MA, USA) was used consisting of a U6K injector, a model 6000A solvent delivery system, and a model 440 absorbance detector operated at 254 nm. A stainless steel Waters separation column (PN 27324 S/N, serial number 106114, packed with microBondapak C18; 300 × 3.9 mm inner diameter) was used. The mobile phase consisted of acetonitrile and a perchlorate solution (44:56). The perchlorate solution (pH 2.5) was prepared by mixing 0.005 M perchloric acid with 0.045 M sodium perchlorate (9:1). The mobile phase was degassed by filtering through organic-aqueous filter paper primed with methanol. All assays were performed at a solvent flow rate of 1 ml/min.

Standard solutions of AMI, nortriptyline (NOR), and the internal standard were prepared in 0.005 M sulfuric acid. Standard curves were run immediately prior to assaying experimental samples and were determined using samples prepared by spiking 1 ml drug-free brain tissue homogenate with known concentrations of AMI and NOR (100, 300, 500, 750, 1000, 1250 ng/ml). Because the brain samples were diluted 1:4, the standards corresponded to experimental samples in concentrations of 500–6250 ng/g brain tissue. These standards were analyzed using the method described above. The drug concentration was determined by comparing the peak height due to the TCA present in the sample to the peak height of the added internal standard, 2-(dibenz[b,5]azepin-5-yl)-N-methylmethylamine (Ciba-Geigy, Basel, Switzerland). Both AMI and its major metabolite NOR were measured in experimental samples. These values were added together to give the total TCA brain concentration.

A heparinized sample of trunk blood and the brains from eight ETOH only and nine ETOH + AMI-treated rats were assayed for ethanol using gas chromatography. The right half of the brain from the ETOH + AMI-treated rats was used for the AMI assay and the left half for the ethanol assay. Plasma and brain ethanol concentrations were determined by gas chromatography using 2-propanol as an internal standard (Burger 1968; Lindsay-Smith and Waddington 1968; Gough and Simpson 1970).

**Results**

**Rotorod.** The administration of ETOH followed by AMI, or ETOH alone, reduced the motor performance of rats on both rotorod tasks when compared with the control group [10 cm $F (3,52) = 12.2, P < 0.001$; 5 cm $F (3,52) = 17.8, P < 0.001$; Fig. 1]. Although AMI-treated rats did not differ from controls in overall performance, there was a negative correlation between the total TCA brain concentration (log) and the performance score for both rotorod tasks (10 cm, $r = -0.72, P < 0.001$; 5 cm, $r = -0.67, P < 0.001$). This concentration-response relationship was also observed when rotorod performance was plotted as a function of total TCA brain concentrations using the pooled results for both rats who received only AMI and rats who were pretreated with ETOH and then given AMI (Fig. 2).

**Open Field.** Rats that received ETOH prior to receiving AMI were also less active in the open field than controls $[t (27) = 2.7, P < 0.02$; Fig. 1]. None of the other groups differed from controls or each other, although total activity for AMI-treated rats was reduced. No correlation was found between the total TCA brain concentration and the open field activity score.

**Drug Assay.** The total TCA brain concentration in rats treated only with AMI was 1857 ± 213 ng/g, whereas the total TCA brain concentration in rats pretreated with ETOH and then given the same dose of AMI was 4141 ± 781 ng/g $[t (25) = 1.86, P < 0.05$, Student’s one tailed t-test]. The parent com-