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A TIME-DEPENDENT BIPHASIC EFFECT OF AN ACUTE ETHANOL INJECTION ON 3-METHOXY 4-HYDROXYPHENYLETHYLENE GLYCOL SULFATE IN RAT BRAIN

BRIAN R. SMITH,* CARLOS M. G. ARAGON and ZALMAN AMIT
Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada

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Abstract—The present experiment demonstrated that acute administration of ethanol appeared to have a biphasic effect on the accumulation of 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO_4) in rat brain. The magnitude of these alterations in MHPG-SO_4 levels was also observed to be highly correlated with peripheral blood ethanol levels. Since levels of MHPG-SO_4 are considered to be an index of noradrenergic activity, the findings suggest that ethanol may affect norepinephrine activity in a specific dose- and time-dependent manner. These results are discussed in reference to previous reports describing apparent divergent effects on norepinephrine. Possible mechanisms for the biphasic actions are also suggested.

Central catecholamines have been implicated in the mediation of the psychopharmacological effects of alcohol. Particular emphasis has been placed on the involvement of norepinephrine (NE) in mediating those effects. Manipulations of central catecholamines, with the neurotoxin 6-hydroxydopamine, demonstrated the importance of NE in the mediation of voluntary ethanol consumption [1–3]. In addition, blockade of NE synthesis has been shown to attenuate ethanol self-administration [4, 5] and voluntary ethanol consumption [6].

Studies examining the effects of ethanol on NE activity have yielded differential findings. Several reports have indicated that alcohol increases the activity [7, 8] and synthesis of brain NE [9], while others have reported a decrease in NE activity [10] and turnover following ethanol administration [11–13]. Most of these studies have used various doses of ethanol with single time measurements, and these apparent opposite effects may be related to the dose-dependent biphasic actions of ethanol [14], as well as possible time-dependent effects [8, 14].

The accumulation of brain 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO_4), a norepinephrine metabolite, has been shown to be an accurate index of central noradrenergic activity [15, 16]. It has also been shown that MHPG-SO_4 concentrations in lumbar cerebrospinal fluid are elevated in healthy intoxicated humans [17], thereby providing further indication for the influence of ethanol on brain noradrenergic mechanisms.

The purpose of the present study was to provide a systematic analysis of the time-dependent actions of ethanol on central noradrenergic activity through measurements of brain MHPG-SO_4 and blood ethanol at various time intervals following acute ethanol administration.

MATERIALS AND METHODS

Male Wistar rats (Charles River Canada, Inc.) weighing 200–225 g were used in the experiment. The animals were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and a 12-hr light–dark cycle. Food and water were available *ad lib*. The animals were given 5 days to acclimatize to the colony conditions and were handled daily prior to the start of the experiment.

Each animal received an intraperitoneal (i.p.) injection of either 2 g/kg of ethanol (8.5 ml/kg of 30% v/v) or Ringer's solution. The animals were killed 5, 10, 15, 30, 60 or 120 min following the i.p. injection, by decapitation. Animals treated with Ringer's solution received i.p. injections in volumes equal to those given to animals receiving ethanol; these control rats were decapitated at the same time intervals as the ethanol-injected animals. Trunk blood was collected for gas chromatographic analysis of blood ethanol levels using the procedures outlined below. The brains were rapidly extracted, rinsed in ice-cold saline, and then frozen on dry ice. The brain samples were stored at −70° and then fluorometrically assayed for MHPG-SO_4 levels.

Determination of MHPG-SO_4. The procedure used was the fluorometric method described by Kohno *et al.* [18]. Briefly, whole brains were homogenized in 5 vol. of 0.2 N H_2SO_4, containing 0.1% sodium metabisulfite ($Na_2S_2O_5$) as a protective agent. The homogenate was then centrifuged at 8000 *g* for 5 min in the cold. The supernatant fraction was divided into three portions (approximately 1/6 to 1/8

* Address all correspondence to: Brian R. Smith, Ph.D., Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 1455 de Maisonneuve Blvd., H-1013, Montreal, Quebec, Canada H3G 1M8.

each) and these were transferred to polycarbonate tubes. One portion was used as a tissue blank and one served as the internal standard. Fifty nanograms of standard MHPG-SO_4 (Tridom Chemicals, Inc.) was added to these tubes. Each sample was adjusted to a pH of 6.0 to 6.5 with 0.3 N and 0.03 N $Ba(OH)_2$. Following a second centrifugation at 8000 *g* for 10 min, the supernatant fraction was decanted into a glass tube and passed through a 0.6 × 6 cm column of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals). The column was washed with 2 ml of distilled water, followed successively by 8 ml of 0.06 N HCl and 1 ml of 0.4 N perchloric acid (PCA). MHPG-SO_4 was eluted from the column with 2 ml of 0.4 N PCA containing 0.1% $Na_2S_2O_5$. Immediately after the separation of MHPG-SO_4, 0.1 ml of 1% cysteine and 0.1 ml of 70% PCA were added to the eluate. These reaction mixtures were heated in an oven at 100° for 30 min. The same reagents were added to the tissue blanks; however, they were not heated. All samples were reheated again for 10 min following the addition of 0.3 ml of freshly distilled ethylenediamine, and then they were cooled in a water bath. The fluorescence was read at wavelengths of 325 nm, excitation, and 465 nm, emission, using an Aminco–Bowman spectrofluorophotometer. Standard values were obtained by subtracting tissue blank and test sample from the internal standard tube readings. This provided the basis for calculations which were calibrated to the known amount of standard added to each tube.

Determination of blood ethanol. The procedure used was based on that of Stowell [19] with modifications from Eriksson *et al.* [20]. These modifications were used to ensure accurate ethanol levels. Animal trunk blood was collected into test tubes which contained 150 I.U. of sodium heparin. Blood (1 ml) was mixed with 4 ml of ice-cold semicarbizide reagent. This mixture was spun in a refrigerated centrifuge at 400 *g* to separate blood cells from serum. Serum (2 ml) was added to 0.5 ml of 3.0 M PCA and then spun at 11,000 *g* to obtain a clear, protein-free supernatant fraction. At this time, 0.5 ml of the supernatant fraction was pipetted into an 8-ml vial, stoppered, and stored at −70° until assayed for ethanol by head-space chromatography [20].

RESULTS

It was observed that the time of decapitation of animals treated with Ringer's solution had no effect on the levels of MHPG-SO_4 in brain. Therefore, the data from these animals were combined into one group for purposes of statistical analysis. Figure 1 represents the effects of an intraperitoneal injection of ethanol on MHPG-SO_4 accumulation in rat brain. A one-way analysis of variance revealed a significant change in the levels of MHPG-SO_4 following ethanol administration [$F(6,73) = 20.64$, $P < 0.001$].

Dunnett's test for comparison involving a control mean [21] demonstrated that there was a significant increase in MHPG-SO_4 levels at 5 ($P < 0.05$), 10 ($P < 0.01$) and 15 min ($P < 0.05$) following the ethanol injection. This was followed by a significant decrease at the 30-min time interval ($P < 0.01$). There were no differences observed at 60 and 120 min as the MHPG-SO_4 levels had returned to control values at these times.

The levels of ethanol in blood following a 2 g/kg injection can be seen in Fig. 2. A one-way analysis of variance indicated that there was a significant alteration in the levels of ethanol over the six time periods examined [$F(5,54) = 19.54$, $P < 0.001$].

Post hoc Tukey tests revealed that peak levels of blood ethanol occurred at 30 min followed by a slight decline to the 120-min time sample.

Correlation coefficients were calculated between

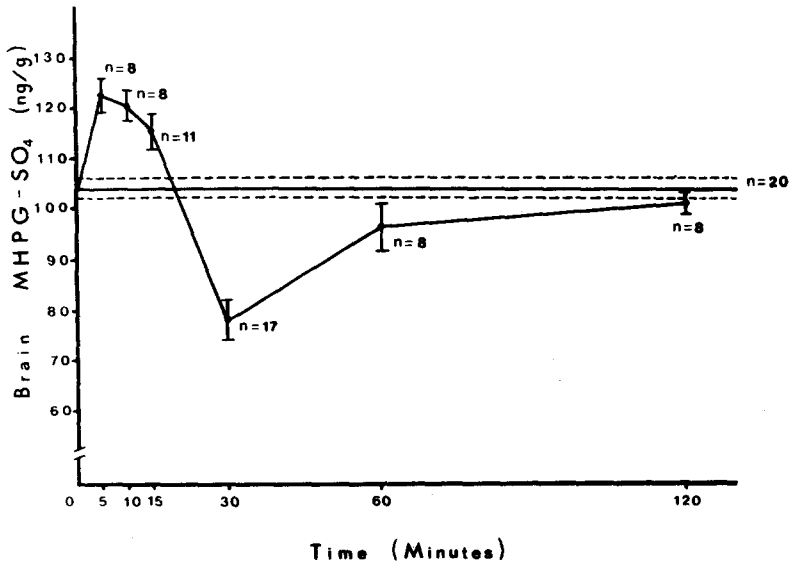

Fig. 1. Mean levels of MHPG-SO_4 in rat brain at 5, 10, 15, 30, 60 and 120 min following an acute ethanol injection (i.p.; 2 g/kg). Vertical lines represent S.E.M. Horizontal line represents control levels with S.E.M. indicated by dotted lines.

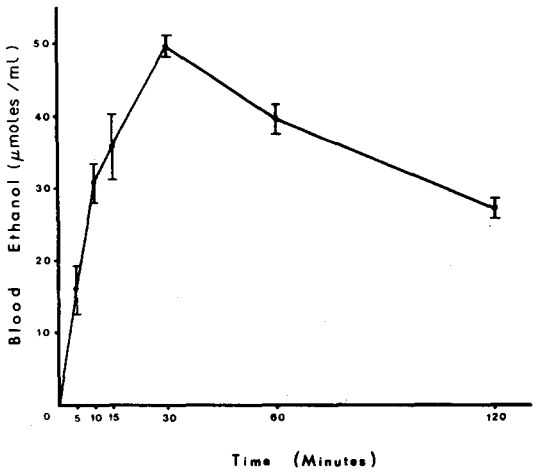

Fig. 2. Mean levels of blood ethanol at various time sampling intervals following an acute 2 g/kg ethanol injection.

the levels of MHPG-SO_4 in brain tissue and the level of ethanol in peripheral blood at the various time intervals sampled. Figure 3 shows the scatter plots for the six time intervals.

The correlations obtained for the first five sampling intervals were statistically significant ($P < 0.05$). Only the data collected at 120 min failed to demonstrate a correlation. These results indicate the presence of an interrelationship between peripheral blood ethanol levels and central levels of MHPG-SO_4. These associations appear to be qualitatively different as the correlations at 5, 10 and 15 min were positive while those at 30 and 60 min were negative.

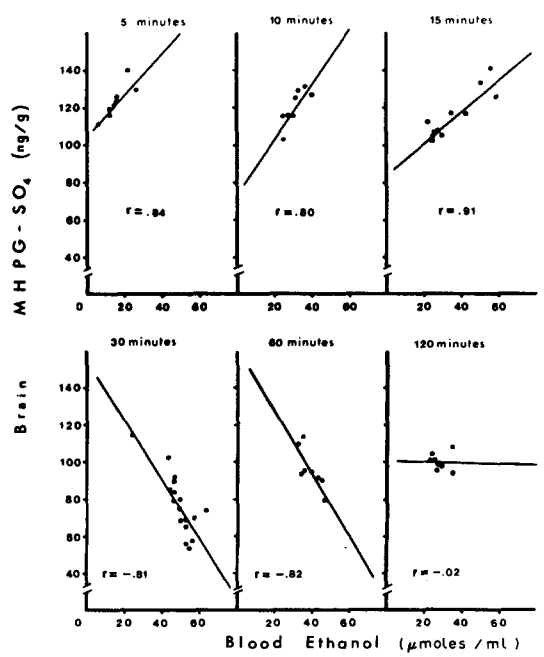

Fig. 3. Scatter plots and correlation coefficients between brain MHPG-SO_4 levels and blood ethanol levels at various time intervals following ethanol administration.

DISCUSSION

The present experiment demonstrated that acute ethanol administration can alter MHPG-SO_4 levels in rat brain and that the magnitude of these alterations is highly correlated with the levels of circulating blood ethanol. The nature of the relationships between brain MHPG-SO_4 and ethanol indicate the presence of a biphasic effect. Central MHPG-SO_4 levels were observed to increase at 5, 10 and 15 min following ethanol administration and then subsequently decrease at 30 min. While ethanol was still detected in the blood at 60 and 120 min following the injection, it appeared that noradrenergic activity returned to baseline as no differences were observed in MHPG-SO_4 levels at these time intervals.

In addition to the alterations seen in the levels of MHPG-SO_4 over time, the qualitative nature of the correlations observed also indicate the presence of a biphasic effect. Correlations at 5, 10 and 15 min were positive while those at 30 and 60 min were negative, indicating a change in the direction of the association between ethanol and MHPG-SO_4.

As indicated earlier, the accumulation of MHPG-SO_4 in brain has been shown to reflect central noradrenergic activity [15, 16]. The present findings confirm previous results indicating that ethanol seems to exert a biphasic effect on NE [8]. The magnitude of this effect appears to be strongly related to the levels of ethanol in the blood; therefore, the nature of the alterations produced by ethanol on the noradrenergic system seems to be not only time dependent but also concentration dependent. This may account for the apparent divergent results observed by other investigators [10–13] as both the concentration of ethanol in the blood and the time following its administration appear to be important in determining the nature of this effect on NE.

The results also reveal the presence of a highly significant relationship between peripheral blood ethanol levels and their central effect on noradrenergic activity as measured by the accumulation of MHPG-SO_4. This was observed at five of the time intervals examined. Only at 120 min following ethanol administration was no correlation observed. As there was no observed difference in MHPG-SO_4 levels at this latter time interval, this may reflect a return to equilibrium of the noradrenergic system. The notion that the observed changes in central NE activity are mediated by peripheral blood ethanol levels is supported by a previous report [22] on observed equilibrium between blood and brain ethanol levels within 3 min following an i.p. injection.

The mechanism by which ethanol may exert two directly opposite effects remains unclear. The decrease in noradrenergic activity may be a compensatory mechanism in response to the initial increase, perhaps mediated by changes in receptor sensitivity [23, 24] or due to possible direct membrane effects of ethanol [25]. Recently, it has been suggested that ethanol preferentially activates noradrenergic neurons and that this may account for the observed stimulatory effects of low doses of ethanol; however, as ethanol levels increase, there may be

greater activation of γ-aminobutyric acid (GABA) neurons resulting in a depressant action [26].

An alternative explanation may conceivably involve the primary metabolite of ethanol, acetaldehyde. Acetaldehyde has been implicated in the mediation of several psychopharmacological actions of ethanol [27]. Since acetaldehyde was reported to trigger the release of central NE [28], it is possible that some interaction between ethanol and acetaldehyde may account for these biphasic effects. Clearly, further research is necessary to elucidate the mode of action of ethanol's effect on norepinephrine and to determine the significance of these interactions and their influence on the behavioral responses to ethanol.

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