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BRIEF COMMUNICATION

Ethanol Differentially Affects Extracellular Monoamines and GABA in the Nucleus Accumbens

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HEIDBREDER, C. AND P. DE WITTE. *Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens.* PHARMACOL BIOCHEM BEHAV **46**(2) 477–481, 1993.—The magnitude, direction, and time course of the effects of acute administration of ethanol (0.1 or 1.0 g/kg) on the extracellular levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and GABA within the nucleus accumbens of the rat were analysed using microdialysis in conjunction with high-pressure liquid chromatography and electrochemical detection. IP injection of 0.1 g/kg ethanol failed to modify the extracellular concentration of DA, DOPAC, HVA, 5-HT, 5-HIAA, and GABA in the nucleus accumbens during the 120-min collection period. On the contrary, 1.0 g/kg IP ethanol significantly increased extracellular levels of DA, DOPAC, and 5-HIAA in microdialysates from the nucleus accumbens. The maximal increase in DA (314.87 ± 6%), DOPAC (210.58 ± 3%), and 5-HIAA (250.88 ± 8%) was observed 40 min after administration of ethanol. Extracellular HVA was also enhanced (220.89 ± 2%) at time point 100 min following injection of the same dose of ethanol. Finally, extracellular levels of 5-HT and GABA remained unchanged following 1.0 g/kg ethanol.

Microdialysis Nucleus accumbens Ethanol Dopamine Serotonin GABA

THERE is now substantial evidence to indicate that activation of the mesolimbic dopamine (DA) system and the resulting positive reinforcing effects seem to determine both a drug's potential for abuse and the addiction process that may subsequently ensue. The release of DA in the nucleus accumbens appears to be essential for the rewarding properties of a variety of abused substances, including ethanol (1,6,20,21).

Systemic administration of ethanol has been reported to activate the synthesis, turnover, or release of DA in the rat whole brain and striatum (3–5,9,10,13,16). More recently, brain microdialysis techniques have enabled the quantitation of neurotransmitter substances and their metabolites in the extracellular fluid from neuroanatomic sites over a time course of several hours. These studies have revealed that systemic administration of ethanol increases the extracellular concentrations of 3,4-dihydroxyphenylethylamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), or homovanillic acid (HVA) in both the nucleus accumbens and dorsal nucleus caudatus of the rat (8,23). Tissue levels of 5-hydroxyindoleacetic acid (5-HIAA) have been reported to be increased in the posterior striatum and nucleus accumbens following systemic administration of high doses of ethanol (10,13) whereas extracellular levels of serotonin [5-hydroxytryptamine (5-HT)] and 5-HIAA in microdialysates from the striatum and nucleus accumbens were also increased for doses up to 2.5 g/kg (23). Local perfusion of ethanol through the microdialysis probe has been shown to markedly increase the extracellular levels of DA, DOPAC, 5-HT, and 5-HIAA in the striatum and nucleus accumbens (22,23). Finally, ethanol induces either no change (18) or an increase in the brain levels of GABA (15) and enhances the number of low-affinity GABA receptors (11,19).

The present study was conducted to test the magnitude, direction, and time course of the effect of acute ethanol treatment on the extracellular levels of DA, DOPAC, HVA, 5-HT, 5-HIAA, and GABA in the nucleus accumbens of the rat.

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METHOD

Subjects and Surgical Procedure

Male Wistar rats weighing 300–350 g were housed in a temperature- and humidity-controlled environment. They had free access to food pellets and water and were kept on a 12 L : 12 D cycle. Animals were anesthetized with chloral hydrate (400 mg/kg, IP) and administered maintenance doses each hour to maintain an approximately constant level of anesthesia throughout the experiment. Each rat was mounted in a stereotaxic frame (David Kopf, Topanga, CA) with the upper incisor bar set 3.5 mm below the interaural line. The skull was exposed and a hole was drilled for unilateral placement of the dialysis probe, which was implanted into the nucleus accumbens according to the following coordinates: rostral, 1.2 mm; lateral, 1.2 mm; ventral, 6.7 mm (14).

Brain Microdialysis Procedure

The microdialysis probes (Carnegie Medicin, 2 mm membrane length) were connected to a microinfusion pump (Harvard 22, Harvard Apparatus, South Natick, MA) and continuously perfused at 1 μl/min with a dialysis buffer containing 5 mM KCl, 120 mM NaCl, 1.2 mM $MgCl_2$, and 1.8 mM $CaCl_2$ for monoamines. The dialysis perfusion buffer for GABA (5 mM KCl, 120 mM NaCl, 2.4 mM $CaCl_2$, and 1.2 mM $MgCl_2$) was delivered at 2.13 μl/min via the same infusion pump. Perfusates were collected every 20 min in microcentrifuge tubes connected to the outlet cannula. Each tube contained 10 μl 0.1 M $HClO_4$ to prevent air oxidation of the monoamines.

Dialysis samples for GABA were collected in tubes containing 10 μl of 5 μM 5-aminovaleric acid (internal standard) in 0.1 M $HClO_4$.

Once monoamine and GABA levels in the perfusates had stabilized (80–100 min), nine consecutive samples were collected every 20 min for the determination of basal levels. Rats were then injected IP with an equal volume of either saline or 10% (v/v) ethanol (Merck, Darmstadt, Germany) in 0.9% saline at doses of 0.1 or 1.0 g/kg body wt., and 20-min dialysis samples were collected for another 120 min.

Chromatographic Analysis of Brain Microdialysates

A chromatography workstation (Maxima 820, Millipore, Ventura, CA) was used in conjunction with an LDC Consta-Metric 3200 solvent delivery pump and a coulometric electrochemical detection system utilizing a preinjection guard electrode (+0.40 V), a preoxidation electrode (+0.10 V), and a working electrode (+0.35 V) for the detection of DA, DOPAC, HVA, 5-HT, and 5-HIAA.

GABA was determined by *o*-phthaldialdehyde (OPA) derivatization. After 67.1 mg OPA was dissolved in 50 ml high-performance liquid chromotography (HPLC)-grade methanol, the solution was diluted with 35 ml HPLC-grade water followed by 15 ml of 1.0 M sodium bicarbonate buffer, pH 9.6. This reagent was then filtered and 56 μl tert-butylthiol was added. For GABA measurement, 40 μl of dialysate was added to 80 μl OPA reagent, allowed to react for 5 min, and then injected onto the HPLC system. The preinjection guard electrode was set at +0.70 V, the preoxidation electrode at +0.20 V, and the analysis electrode worked at +0.39 V. A six-port rotary valve (Model 7125, Rheodyne, Berkeley, CA) was used for sample injection. Chromatographic separations were performed using a 100 mm × 3.2-mm i.d. stainless steel column packed with octadecylsilane (C18) on microparticulate (3 μm) silica gel (Spherisorb ODS).

The mobile phase for the separation of catecholamines and their metabolites was a mixture of 0.1 M citrate, 0.075 M Na_2HPO_4, 1.2 mM sodium heptanesulfonate, 0.097 mM EDTA, and 13% methanol (v/v), adjusted to pH 4.0. The mobile phase for the determination of serotonin consisted of 0.15 M NaH_2PO_4, 0.01 mM sodium octanesulfonate, 0.5 mM EDTA, and 12% methanol (v/v), adjusted to pH 3.9. Finally, the mobile phase for GABA consisted of 55 : 45 0.1 M Na acetate buffer : acetonitrile, adjusted to pH 4.85.

All mobile phases were filtered through a 0.2-μm filter (Gelman Sciences, Ann Arbor, MI), degassed under vacuum, and delivered at a flow rate of 0.70 ml/min. The position and height of the peaks of the endogenous components were compared with 30-μl samples of a standard solution containing 10^{-8} M DA, 10^{-7} M DOPAC, HVA, 5-HT, 5-HIAA, and GABA. All reagents were analytic grade and were obtained from Sigma Chemical Co. (St Louis, MO).

Histology

After the final dialysis sample was collected, the rat was sacrificed and the brain fixed with 10% formalin. For histological verification of probe placement, coronal sections (100 μm thick) were made with a microtome (Polaron H1200, Bio-Rad, Cambridge, MA) and stained with 0.5% cresyl violet. Dialysis probe placement was localized according to the atlas of Paxinos and Watson (14). Of the 72 rats used in the present study, 11 were eliminated from the data analysis due to histological visualization of the microdialysis probe outside the nucleus accumbens. In five cases, the probe was found to reach the caudate putamen. In four other cases, the probe was implanted in the ventral part of the lateral septal nucleus. Finally, two rats were implanted in the ventral pallidum.

Analysis of Data

Only data from animals with histologically correct cannulae placements were used for subsequent statistical analysis. The data are expressed as percentages of the mean value of the three basal samples before administration of ethanol or saline.

Dose–response curves were analysed using a two-way analysis of variance (ANOVA) (treatment × time) with repeated measures on one factor. The posthoc two-tailed Dunnett's *t*-test was used to determine statistical significance between treatment and control (saline injected) values. The accepted level of significance for all tests was $p < 0.05$.

RESULTS

Mean basal levels of the monoamines, their metabolites, and GABA in the nucleus accumbens were (in pmol/30 μl of perfusate): 0.21 ± 0.04 for DA, 10.67 ± 2.3 for DOPAC, 9.17 ± 1.6 for HVA, 0.14 ± 0.03 for 5-HT, 12.03 ± 1.42 for 5-HIAA, and 0.48 ± 0.09 for GABA.

IP administration of saline 0.9% did not alter the extracellular level of DA, DOPAC, 5-HT, 5-HIAA, and GABA at any time points (Fig. 1).

Ethanol (0.1 g/kg, IP) remained without any significant statistical effect on the extracellular concentration of DA, DOPAC, HVA, 5-HT, 5-HIAA, and GABA in the nucleus accumbens during the 120-min collection period (Fig. 2).

On the contrary, ethanol (1.0 g/kg, IP) significantly elevated extracellular concentrations of DA, DOPAC, and 5-

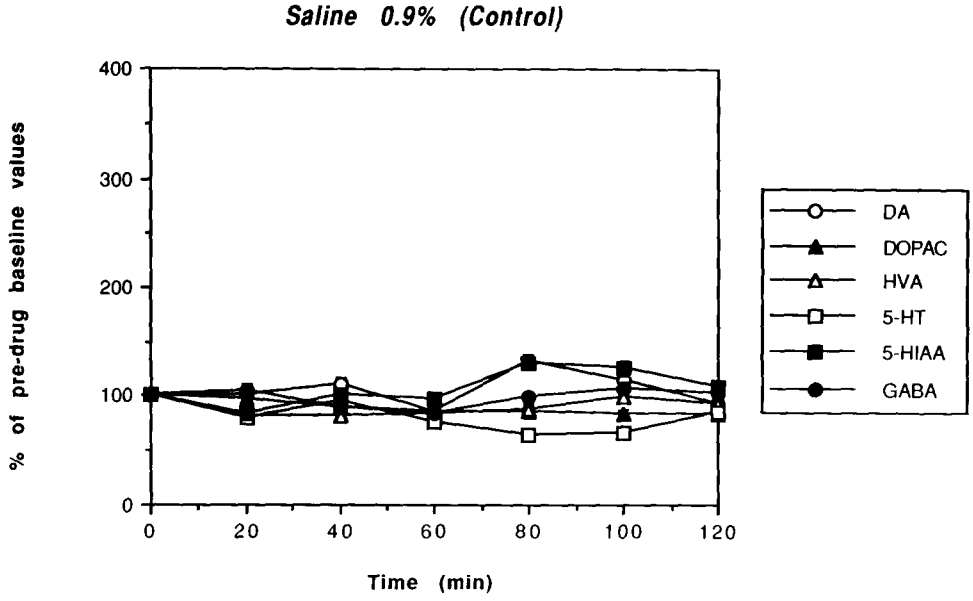

FIG. 1. Effects of saline 0.9% on the extracellular concentration of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and GABA in microdialysates from the nucleus accumbens of the rat. Concentrations are shown as the percent changes from the average of the three baseline samples obtained prior to IP injection of saline 0.9%. Data are the means ± SEM (n = 21).

HIAA in microdialysates from the nucleus accumbens (Fig. 3). The maximal increase in DA (314.87 ± 6%), DOPAC (210.58 ± 3%), and 5-HIAA (250.88 ± 8%) was observed 40 min after ethanol treatment. Extracellular HVA was also enhanced (220.89 ± 2%) at time point 100 min following administration of the same dose of ethanol. Finally, extracellular levels of 5-HT and GABA remained unchanged following 1.0 g/kg ethanol.

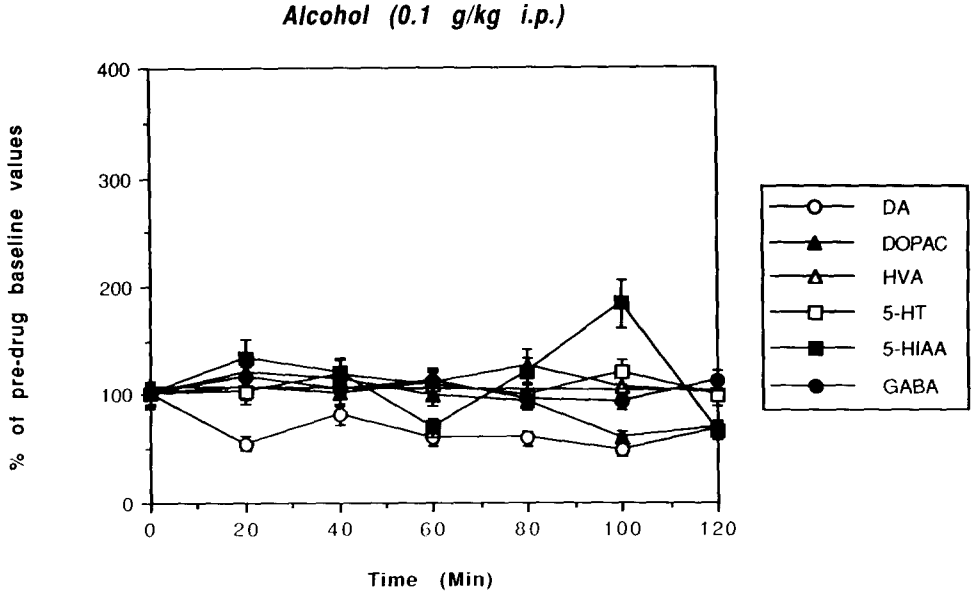

FIG. 2. Effects of ethanol on the extracellular concentration of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and GABA in microdialysates from the nucleus accumbens of the rat. Concentrations are shown as the percent changes from the average of the three baseline samples obtained prior to IP injection of 0.1 g/kg ethanol. Data are the means ± SEM (n = 19).

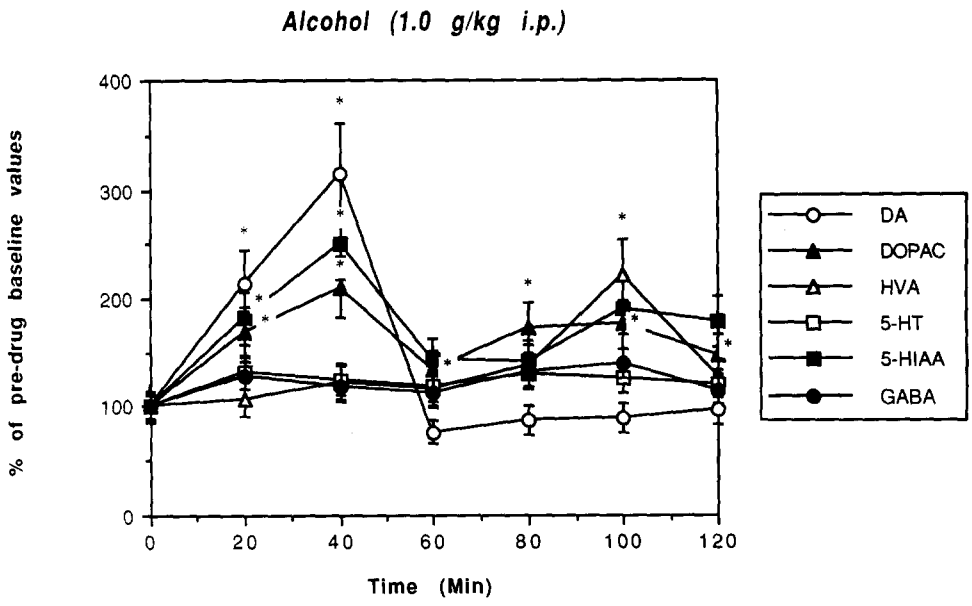

FIG. 3. Effects of ethanol on the extracellular concentration of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and GABA in microdialysates from the nucleus accumbens of the rat. Concentrations are shown as the percent changes from the average of the three baseline samples obtained prior to IP injection of 1.0 g/kg ethanol. Data are the means ± SEM (n = 21). *$p < 0.05$, comparing ethanol to saline using a two-way analysis of variance with repeated measures on one factor, followed by a two-tailed Dunnett's t-test.

DISCUSSION

Our results indicate that acute administration of ethanol using a dose of 1.0 g/kg increased the extracellular concentrations of DA, DOPAC, HVA, and 5-HIAA in the nucleus accumbens of the rat. On the contrary, ethanol remained without any statistically significant effect on the levels of 5-HT and GABA in the same structure.

Ethanol stimulates the firing rate of neurons in the ventral tegmental area (2,7) and increases the metabolism of DA in the nucleus accumbens of the rat (10,13). A dose of 1.0 g/kg IP of ethanol has been reported to significantly enhance the release of DA in the nucleus accumbens by about 100% 40 min after administration (8). Although less than DA, the extracellular levels of DOPAC and HVA have also been shown to be increased 40 and 60 min following injection of the same dose of ethanol (8). More recently, Yoshimoto et al. (23) demonstrated that 1.0 and 2.0 g/kg ethanol increased the extracellular levels of DA in the nucleus accumbens with only a small increase in the concentration of HVA following the highest dose of ethanol; these results are more related to the data reported for the caudate nucleus (8). The local perfusion of 100 mM ethanol through the microdialysis probe markedly increased the extracellular levels of DA, 5-HT, DOPAC, and 5-HIAA (23) and the local perfusion of ethanol at concentrations ranging from 157.6 to 788.0 mM was also shown to induce an increase of extracellular DA in the same brain area (22).

Although extracellular levels of 5-HIAA were strongly increased (maximal effect 40 min after 1.0 g/kg ethanol), we failed to observe any modification in the extracellular concentration of 5-HT. These results do not agree with the data of Yoshimoto et al. (23), who found that 1.0 g/kg ethanol induced an increase in the extracellular level of 5-HT without any effect on the concentration of 5-HIAA; this discrepancy might be due to placement of the dialysis probe within the nucleus accumbens.

Our results failed to reveal any modification in the extracellular level of GABA following IP injection of 1.0 g/kg ethanol. GABA has been reported to be involved in regulating the pre- and postsynaptic inhibition of neuronal activity and it has been suggested that the excitatory effects of small doses of ethanol could be the result of inhibition of such a GABA inhibitory system (17), whereas the sedative effects induced by large doses of ethanol could be mediated by activation of this same system. Brain ethanol concentrations 15–30 min after an IP injection of 1.0 g/kg can be approximated from blood ethanol levels (12) and are estimated to be about 20 mM (10) whereas the mean concentration of ethanol in brain tissue following a 20-min perfusion with 157.6 mM ethanol would be 1.35 mM (22). Higher peripheral doses of ethanol seem thus necessary to modify the extracellular level of GABA within the nucleus accumbens.

In summary, our data indicate that systemic administration of 1.0 g/kg ethanol leads to an increase in the basal concentrations of DA, DOPAC, HVA, and 5-HIAA within the nucleus accumbens. The effect of systemic administration of ethanol on the extracellular concentration of GABA within the nucleus accumbens remains to be elucidated.

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