ELSEVIER

European Journal of Pharmacology 301 (1996) 49–57

ejp

Focal ethanol elevates extracellular dopamine and serotonin concentrations in the rat ventral tegmental area

Qing-Shan Yan *, Maarten E.A. Reith, Phillip C. Jobe, John W. Dailey

Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656, USA

Received 28 December 1995; accepted 3 January 1996

Abstract

The present study describes the effects of local perfusion with ethanol on extracellular dopamine and serotonin (5-hydroxytryptamine, 5-HT) concentrations in the ventral tegmental area. Various concentrations of ethanol in artificial cerebrospinal fluid (ACSF) (0.1–10%, v/v) were administered through a microdialysis probe into the ventral tegmental area of freely moving Sprague-Dawley rats. A significant and concentration-dependent increment in dialysate output of both dopamine and serotonin was observed after local infusion of ethanol. Perfusion with Ca^{2+}-free medium or tetrodotoxin (1 μM in ACSF) produced a significant reduction in basal extracellular dopamine and serotonin concentration but failed to block dopamine or serotonin release produced by infusion of 10% ethanol. Perfusion with 100 mM K^+ before and after infusion of 10% ethanol revealed that the second perfusion with high K^+ solution still produced an increase in dopamine and serotonin concentration, similar in magnitude to the first response, indicating that perfusion with 10% ethanol did not cause irreversible damage to either dopamine cell bodies or serotonin terminals in the ventral tegmental area. These results suggest that dopamine and serotonin release from the ventral tegmental area produced by focal application of 10% ethanol is mediated, at least in part, by a non-exocytotic mechanism. Direct stimulation of the ventral tegmental area dopamine neurons by ethanol might be involved in the reinforcing properties of the drug.

Keywords: Ethanol; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; Ventral tegmental area

1. Introduction

A number of physiological, neurochemical and pharmacological studies suggest an important role for mesolimbic dopamine neurotransmission in the reinforcing and intoxicating actions of ethanol. Pharmacological manipulation of dopamine transmission by both systemic and intra-accumbens administration of dopamine receptor agonists and antagonists alters ethanol preference and oral self-administration patterns (Pfeffer and Samson, 1988; Rassnick et al., 1993; Weiss et al., 1990). Consistent with a mesolimbic dopaminergic role in the regulation of the voluntary intake of ethanol, a recent investigation by Lanca (1994) revealed that dopamine-rich brain grafts, transplanted into the ventromedial striatum of adult rats, significantly reduced the voluntary consumption of alcohol. Furthermore, alcohol-induced behavioral activation is closely time-locked to stimulation of dopamine release in the nucleus accumbens and both the behavioral and dopaminergic effects are blocked by γ-butyrolactone, a compound that inhibits dopamine neuronal activity and release (Imperato and Di Chiara, 1986). In view of the close relationship between locomotor stimulation and reward potential (Wise and Bozarth, 1987) these findings suggest that the locomotor-enhancing effects of ethanol reflect the reinforcing properties of this drug and that both the locomotor and acute reinforcing of actions of ethanol are the result of its ability to stimulate dopamine release in the nucleus accumbens.

In addition, there is abundant documentation concerning the effects of ethanol on the serotonergic system. The systemic or local administration of ethanol increases extracellular concentration of serotonin (5-hydroxytryptamine, 5-HT) in the nucleus accumbens of Wistar rats (Yoshimoto et al., 1991), high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) lines of rats (Yoshimoto et al.,

* Corresponding author. Fax: 309-671-8403.

PII S0014-2999(96)00018-0

1992). In Sardinian alcohol preferring rats, Portas et al. (1994) have demonstrated that ethanol administration produced a significant increase in cortical serotonin efflux.

The ventral tegmental area contains the A10 group of dopamine neurons and is the origin of most of the dopaminergic fibers that innervate cortical and limbic structures, such as the nucleus accumbens and prefrontal cortex (Oades and Halliday, 1987). Studies of the afferent pathways to the ventral tegmental area have indicated that this region receives serotonergic innervations from the dorsal and median raphe nuclei (Moore et al., 1978), as many as 50% of the serotonergic terminals in the ventral tegmental area make direct synaptic contacts on both dopamine and non-dopamine cells (Herve et al., 1987). Moreover, the ventral tegmental area has been shown to have high levels of serotonin (Ruggeri et al., 1987) and possess high affinity serotonin uptake sites (Beart and McDonald, 1982). A Ca^{2+}-dependent, K^+-stimulated [^{3}H]serotonin release has also been demonstrated in slices of the ventral tegmental area (Beart and McDonald, 1982).

Although the effect of ethanol on dopamine transmission in the dopamine terminal regions appears to be the basis of many ethanol's actions, the alterations in monoamine activity in the ventral tegmental area might also be responsible for the occurrence of a behavioral response to ethanol. Indeed, dopamine neurons in the ventral tegmental area have been implicated in cognitive and emotive functions (Simon et al., 1980), in the mediation of the reinforcing properties of drugs of abuse such as cocaine (Roberts and Koob, 1983; Chen and Reith, 1993b, 1994b), amphetamine (LeMoal et al., 1979; Lyness et al., 1979), heroin (Bozarth and Wise, 1981), morphine (Gysling and Wang, 1983; Matthews and German, 1984) as well as in the reward induced by the electrical brain self-stimulation (Fouriezos and Wise, 1976; Wise, 1978). However, there is no information in the literature on the effect of ethanol on neurotransmitter release in the ventral tegmental area. Therefore, the investigation of the local effect of ethanol on dopamine release in a dopamine cell body area is an essential step toward understanding the involvement of dopamine neurons in ethanol's action. In view of the fact that the serotonergic system is affected by ethanol and that there are anatomical and functional relationships between dopamine and serotonin systems in the ventral tegmental area, it would also be interesting to investigate the effect of local ethanol on serotonin release in the ventral tegmental area. The present study was designed to address this issue with an intracerebral microdialysis approach in which dopamine and serotonin were measured simultaneously in dialysates from the ventral tegmental area of freely moving rats. Ethanol was applied directly to the ventral tegmental area via the dialysis probe to circumvent pharmacokinetic factors and to minimize the effects of ethanol on non-ventral tegmental area structures that could indirectly influence ventral tegmental area monoamine transmission.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, weighing 280–320 g at the time of surgery, were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA). They were housed at 21 ± 3°C, 40–60% relative humidity and were maintained under 12 h light/12 h dark condition with ad libitum access to food and water before using.

2.2. Drugs

Dehydrated alcohol was obtained from American Reagent Laboratories (Shirley, NY, USA) and diluted with artificial cerebrospinal fluid (ACSF) to 0.1, 1, and 10% (v/v), respectively. Tetrodotoxin was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in ACSF. Both ethanol and tetrodotoxin were administered through the dialysis probes. Reagents used in chemical assays were of analytical grade.

2.3. Microdialysis

The animals were prepared for the microdialysis experiments as described in a previous paper (Yan et al., 1992). In brief, surgery was conducted on a Kopf stereotaxic instrument under anesthesia with a combination of sodium pentobarbital (35 mg/kg, i.p.) and halothane (5% in oxygen). A dialysis guide cannula (Harvard Apparatus, S. Natick, MA, USA) was stereotaxically implanted over the ventral tegmental area and attached to the skull with dental acrylic and machine screws. To avoid rupture of the sagittal sinus when inserting the dialysis probe, the guide, aimed towards the ventral tegmental area, was introduced into the skull at a 14° angle from the sagittal plane. The coordinates relative to bregma were: AP −5.2 mm, L 3.0 mm (Paxinos and Watson, 1986). The period of post-surgical recovery was at least 5 days. On the experimental day, a loop dialysis probe (1.5 mm in length), made from cellulose acetate hollow fibers (I.D. 215 ± 15 μm, molecular weight cutoff = 6000; Spectrum Medical Industries, Los Angeles, CA, USA), was inserted into the guide and directed to the ventral tegmental area with the tip 8.4 mm below the dura while gently restraining awake rats. The animals were then placed individually into a plexiglass chamber where they could move about freely. ACSF, which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155), was perfused at 1.5 μl/min. Consecutive samples were collected throughout 20-min intervals. No treatments were administered until basal release of both dopamine and serotonin were stable. This occurred 3–4 h after probe insertion. In the experiments with ethanol alone, progressively higher concentrations of ethanol (0.1, 1, 10%) were administered via the dialysis probe in order to determine the dose-response relationship.

Each concentration of ethanol was perfused for 60 min. When Ca^{2+}-free medium was used, Ca^{2+} was replaced by Mg^{2+}; and when ACSF with 100 mM K^+ was employed, the concentration of Na^+ in ACSF was adjusted to maintain osmolarity.

The rats were directly connected to high performance liquid chromatography (HPLC) equipment. Two fused silica tubes (inner diameter = 75 μm) were connected to the inlet and outlet of the dialysis probe, respectively. One tube was connected to the perfusion pump via a liquid swivel, and the other to Valco 2 position valves (Valco Instruments Co., Houston, TX, USA). With the help of an electronic timer, the valve was held in the load position for 20 min during which the sample loop (20 μl) was filled with dialysate. The valve was then switched automatically to the injection position for 15 s. This procedure was repeated every 20 min, which was the time needed to record a complete chromatogram. Drug delivery and sample collection time were corrected for the lag time resulting from the dead volume of the inlet and outlet tubes.

2.4. Analytical and histological procedure

Dialysate samples were injected onto a HPLC system with electrochemical detection. This system consisted of an ESA solvent delivery system (model 580), an ESA HR-80 column (3 μm, ODS, 80 × 4.6 mm), and an ESA coulochem II electrochemical detector equipped with a dual electrode analysis cell (M 5014) and a guard cell (M5020). The guard cell was set at 400 mV, electrode 1 at −100 mV, and electrode 2 at 225 mV with respect to palladium reference electrodes. The mobile phase contained 75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 20 μM EDTA, 100 μl/l triethylamine (pH 5.6 with H_3PO4), 12% methanol, and 12% acetonitrile; and was pumped through the system at 1 ml/min. Chromatograms were integrated, compared with standards run separately on each experimental day, and analyzed using a computer-based data acquisition system (Maxima 820, Waters, Milford, MA, USA). The detection limit for dopamine and serotonin was 1 fmol at a 2 : 1 signal-to-noise ratio.

After completion of the dialysis, the animals were decapitated and the brains immersion-fixed overnight in buffered 4% paraformaldehyde. Forty micrometer thick coronal sections were cut on a freezing microtome, stained with neutral red and analyzed in the light microscope. The locations of the dialysis probes were verified in each brain. Data from animals where the probes were not located within the ventral tegmental area were not included in the present study.

2.5. In vitro probe recovery for ethanol

The in vitro probe recovery for ethanol was tested under room temperature. Tracer amounts of ^{14}C-labeled ethanol (New England Nuclear, Wilmington, DE, USA) were added to a 1% solution of cold ethanol in ACSF. The resultant bath solution contained 7800 cpm/μl. The recovery was determined by perfusing four probes placed in the above-mentioned bath solution. ACSF was passed through the probes at a constant flow rate of 1.5 μl/min for 2 h (for equilibrium), and fractions were then collected every 20 min into a scintillation vial containing 5 ml of Cytoscint (ICN, Costa Mesa, CA, USA). Vials were vortexed and counted for ^{14}C in a scintillation counter (Beckman LS6000IC, Beckman Instruments, Fullerton, CA, USA). The in vitro probe recovery for ethanol was calculated according to the following equation: recovery = (cpm/μl of dialysate ÷ cpm/μl of bath) × 100%, and was: 12.8 ± 0.3% (mean ± S.E.M., $n = 4$).

2.6. Analysis of data

The in vitro recoveries at 1.5 μl/min of the dialysis probes used in this study were approximately 9% and 10% for dopamine and serotonin, respectively. The monoamine data reported here were not corrected for these recoveries. Changes in extracellular dopamine and serotonin induced by treatments were expressed as percentage of the mean basal output obtained in each individual rat. The dialysis data were analyzed through the use of Student's *t*-test. The accepted level of significance was 0.05.

3. Results

3.1. Effects of local infusion of ethanol on extracellular dopamine and serotonin concentration in the ventral tegmental area

Fig. 1 shows the effects of local perfusion with ethanol on extracellular dopamine (upper panel) and serotonin (low panel) concentrations in the ventral tegmental area. Prior to the treatment, the mean dopamine and serotonin contents in ventral tegmental area extracellular fluid were (fmol/μl of dialysate ± S.E.M.) 0.47 ± 0.05 ($n = 5$) and 1.05 ± 0.10 ($n = 5$), respectively. As can be seen from Fig. 1, ethanol, at 0.1–10%, elicited a significant and concentration-dependent increment in dialysate levels of both dopamine and serotonin without selectivity. There was a tendency toward an increase in extracellular concentrations of dopamine and serotonin after perfusion with 0.1% ethanol, although the effects were not statistically significant. 1% and 10% ethanol caused extracellular dopamine concentration to reach the maximum levels of 187% and 528% of baseline, respectively; while serotonin reached the maximum of 262% and 728% of baseline, respectively.

3.2. Effects of perfusion with Ca^{2+}-free medium on ethanol-induced increases in extracellular content of dopamine and serotonin in the ventral tegmental area

Basal extracellular dopamine and serotonin concentrations were (fmol/μl of dialysate ± S.E.M.) 0.48 ± 0.07

($n = 4$) and 1.24 ± 0.20 ($n = 4$), respectively. Perfusion with Ca^{2+}-free medium caused basal extracellular dopamine (Fig. 2, upper panel) and serotonin (Fig. 2, low panel) concentration to decrease significantly. The maximum reduction in basal dopamine output was 76.2% and that in serotonin output 58.2% of baseline. However, introduction of 10% ethanol into Ca^{2+}-free medium still produced dramatic increases in the dopamine and serotonin outputs. Under these conditions, the maximum levels in extracellular dopamine and serotonin concentration were 374% and 548% of baseline, respectively; and not different from those in response to 10% alcohol alone shown in Fig. 1 ($t = 0.94$, $P > 0.2$, for dopamine; $t = 0.77$, $P > 0.4$, for serotonin).

3.3. Effects of perfusion with tetrodotoxin on ethanol-induced increases in extracellular content of dopamine and serotonin in the ventral tegmental area

Basal output of dopamine and serotonin in the dialysate from the ventral tegmental area was (fmol/μl of dialysate $\pm$ S.E.M.): 0.39 ± 0.06 ($n = 4$) and 0.87 ± 0.15 ($n = 4$), respectively. As shown in Fig. 3, tetrodotoxin (1 μM) infusion caused basal dopamine (upper panel) and serotonin (lower panel) concentration to decrease to 19.3% and 18.3% of baseline, respectively. Although nerve-firing activity was blocked by perfusion of tetrodotoxin, 10% ethanol still increased dopamine and serotonin outputs in the dialysate to 630% and 465% of baseline, respectively. The maximum levels in extracellular dopamine and serotonin concentration after co-administration of tetrodotoxin with 10% ethanol were not different from those in response to 10% alcohol alone shown in Fig. 1 ($t = 0.626$, $P > 0.5$, for dopamine; $t = 1.26$, $P > 0.2$, for serotonin).

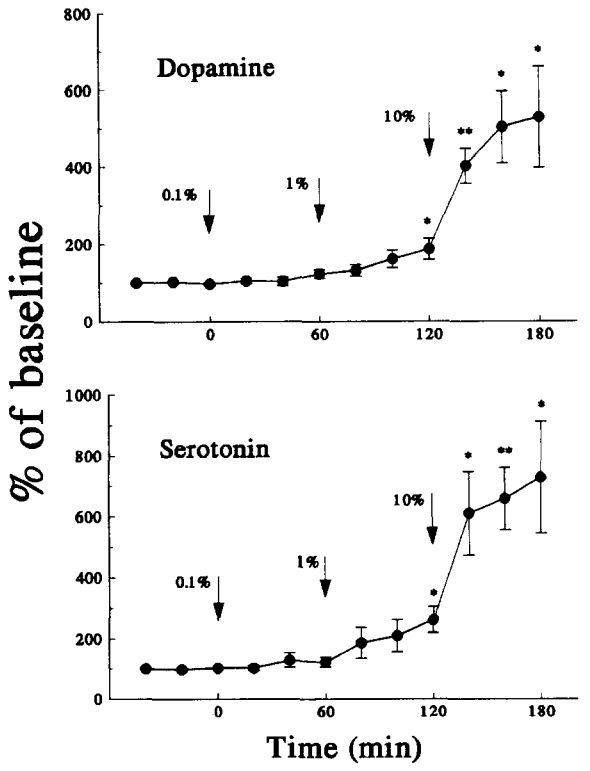

Fig. 1. Effects of local infusion of ethanol on extracellular dopamine (upper panel) and serotonin (lower panel) concentration in the ventral tegmental area of freely moving rats. Ethanol (0.1, 1, 10%; v/v) was administered via the dialysis probes at the times indicated by the arrows. Results are means with S.E.M. of data obtained from 5 rats and expressed as the percentage of the pre-treatment baseline. * $P < 0.05$; ** $P < 0.01$ as compared with pre-treatment baseline. Paired t-test.

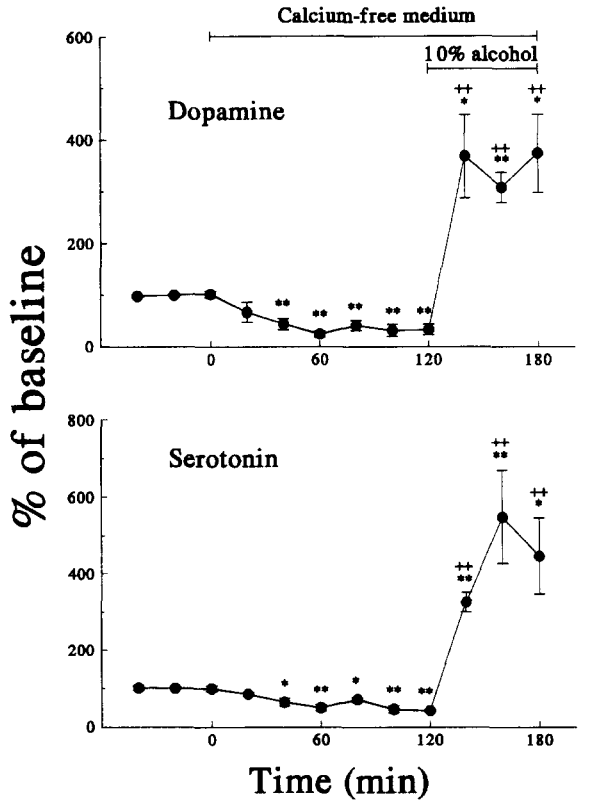

Fig. 2. Effects of perfusion with Ca^{2+}-free medium on ethanol-induced increases in extracellular content of dopamine (upper panel) and serotonin (lower panel) in the ventral tegmental area of freely moving rats. The Ca^{2+}-free medium alone and Ca^{2+}-free medium plus 10% ethanol were administered via the dialysis probes and the periods of perfusion were from time 0 to 180 min and from time 120 to 180 min, respectively (indicated by the bars). Results are means with S.E.M. of data obtained from 4 rats and expressed as the percentage of the pre-treatment baseline. * $P < 0.05$; ** $P < 0.01$ as compared with pre-treatment baseline. $^{++}$ $P < 0.01$ as compared with infusion of Ca^{2+}-free medium alone. Paired t-test.

3.4. Effect of perfusion with high K^+ solution before and after infusion of 10% ethanol on the dialysate levels of dopamine and serotonin in the ventral tegmental area

Prior to the treatment, the mean dopamine and serotonin contents in ventral tegmental area extracellular fluid were

(fmol/μl of dialysate ± S.E.M.) 0.42 ± 0.05 ($n = 4$) and 0.94 ± 0.10 ($n = 4$), respectively. As can be seen from Fig. 4, the first perfusion with 100 mM K^+ solution caused extracellular dopamine and serotonin to increase dramatically to 2209% and 1017% of pre-treatment baseline, respectively. Extracellular dopamine and serotonin concentrations fell rapidly following the discontinuation of high K^+ perfusion and returned to the pre-treatment basal value at 80 min after switching to ACSF infusion. At this time point, perfusion with 10% ethanol caused extracellular dopamine and serotonin concentrations to reach a maximum level of 440% and 570% of pre-treatment baseline, respectively. A second perfusion with 100 mM K^+ solution after extracellular dopamine and serotonin had returned to pre-treatment basal value produced a significant increase in dopamine and serotonin output. The maximum levels for dopamine and serotonin after a second perfusion with high K^+ were 1826% and 1346% of pre-treatment baseline, respectively, and were similar in magnitude to those in response to the first perfusion with 100 mM K^+ solution.

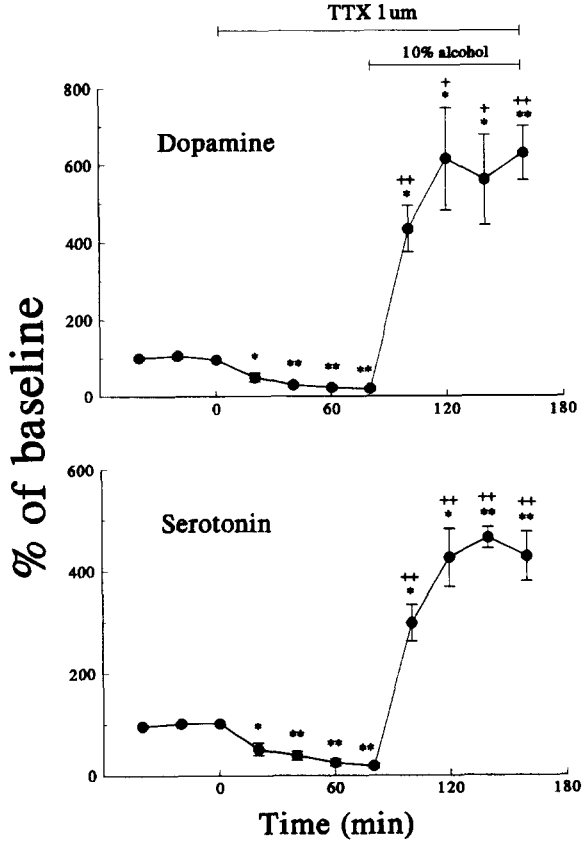

Fig. 3. Effects of perfusion with tetrodotoxin on ethanol-induced increases in extracellular content of dopamine (upper panel) and serotonin (lower panel) in the ventral tegmental area of freely moving rats. The tetrodotoxin alone and tetrodotoxin plus 10% ethanol were administered via the dialysis probes and the periods of perfusion were from time 0 to 160 min and from time 80 to 160 min, respectively (indicated by the bars). Results are means with S.E.M. of data obtained from 4 rats and expressed as the percentage of the pre-treatment baseline. $^{*}P < 0.05$; $^{**}P < 0.01$ as compared with pre-treatment baseline. $^{+}P < 0.05$; $^{++}P < 0.01$ as compared with infusion of tetrodotoxin alone. Paired t-test.

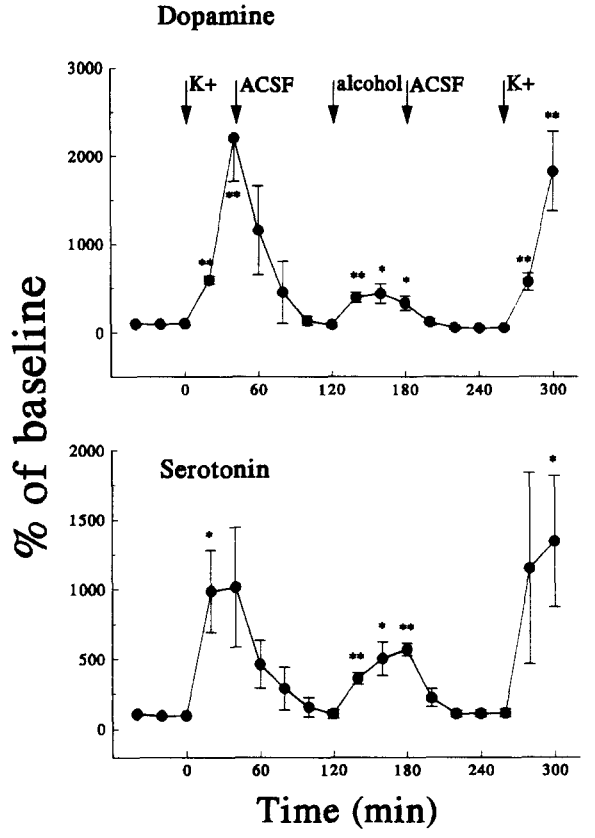

Fig. 4. Effect of perfusion with 100 mM K^+ solution before and after infusion of 10% ethanol on the dialysate levels of dopamine (upper panel) and serotonin (lower panel) in the ventral tegmental area of freely moving rats. One hundred millimolar K^+ solution, ACSF, and 10% ethanol were administered, respectively, as indicated by the arrows. Results are means with S.E.M. of data obtained from 4 rats and expressed as the percentage of the pre-treatment baseline. $^{*}P < 0.05$; $^{**}P < 0.01$ as compared with pre-treatment baseline. Paired t-test.

4. Discussion

To our knowledge, the present study provides the first report on the effects of focally applied ethanol on the extracellular dopamine and serotonin concentrations in the ventral tegmental area, a cell body area of the mesocorticolimbic dopamine system, of freely moving rats. As basal dialysate dopamine and serotonin outputs are significantly inhibited by the blockade of Na^+ channels with tetrodotoxin (decreased to 19.3% and 18.3% of baseline, respectively) and by exclusion of Ca^{2+} from perfusion medium (decreased to 23.8% and 41.8% of baseline, respectively), most of dopamine and serotonin in the dialysate from the ventral tegmental area observed in this investigation are action potential-dependent and, therefore, of neu-

ronal origin. This finding is in agreement with previous observations which showed that dopamine release from somatodendritic regions is a physiological event, and that this type of release also fulfills several classical neurotransmitter criteria such as dependence upon the opening of fast Na^+ channels and autoreceptor regulation (Santiago and Westerink, 1992; Chen and Reith, 1994a, 1994b).

Our study demonstrates that ethanol, when locally administered into the ventral tegmental area via a microdialysis probe, caused a marked increase in the dialysate levels of dopamine in a dose-related fashion. This is in accord with many previous studies which have examined ethanol effects on dopamine release in vitro, ex vivo or most recently in vivo, after systemic and local ethanol administration. Seeman and Lee (1974) showed enhanced spontaneous release of dopamine in preloaded rat caudate synaptosomal preparations following ethanol. Recent microdialysis studies have shown that systemic and local application as well as oral self-administration of ethanol increased extracellular dopamine concentration in the rat striatum (Wozniak et al., 1991) and nucleus accumbens (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991, 1992; Weiss et al., 1993).

As mentioned above, the ventral tegmental area contains dopamine cell bodies projecting to limbic structure such as the nucleus accumbens (Oades and Halliday, 1987). It has been reported that local infusion of ethanol into the nucleus accumbens of Wistar (Yoshimoto et al., 1991) and Sprague-Dawley rats (Wozniak et al., 1991) results in elevated levels of extracellular dopamine. The present study shows that local application of ethanol increases extracellular dopamine concentration in the ventral tegmental area. Taken together, these findings indicate that ethanol increases dopamine output in both cell body and terminal areas of the mesolimbic dopamine pathway when it is focally applied. This view is consistent with the previous observations that ethanol increases the firing rate of the dopaminergic neurons of the ventral tegmental area both in vivo (Gessa et al., 1985) and in vitro (Brodie et al., 1990) over a range of behaviorally relevant concentrations.

The dopamine-containing cells of the ventral tegmental area have been implicated in the mediation of reward produced by a variety of drugs of abuse, including ethanol (Wise, 1987; Samson et al., 1992). Most drugs of abuse increase dopaminergic transmission in the central nervous system, either by stimulating dopaminergic neurons (e.g., opiates) (Klitenick et al., 1992) or by reducing reuptake of dopamine (e.g., cocaine) (Chen and Reith, 1994a, 1994b). Microinjection of quinpirole into the ventral tegmental area reduces responding for ethanol reward (Hodge et al., 1993). Inasmuch as there is much pharmacological evidence suggesting that activation of mesocorticolimbic dopaminergic neurons is important for the rewarding effects of ethanol consumption, our finding of increased dopamine levels in the ventral tegmental area following local perfusion with ethanol provides further support to the concept that the reinforcing properties of ethanol may derive, at least in part, from its ability to stimulate dopamine transmission in the mesolimbic dopamine pathway.

The use of microdialysis in the dopamine cell body regions of the rat allows for the study of somatodendritic release of dopamine, a key mechanism through which dopamine neurons (auto)regulate their activity. It has been accepted that there are at least two types of regulation of dopamine neuron in the ventral tegmental area. The first is called feedback regulation which arises from limbic and cortical dopamine terminal fields and is often termed long-loop feedback because it is regulated by axonal dopamine release. The second is intrinsic regulation. This type of regulation consists of short-loop feedback regulation of dopamine neurons, since it involves somatodendritic dopamine release. A primary function of somatodendritically released dopamine is to provide autoinhibition of impulse generation and somatodendritic dopamine transmission. It has been demonstrated that systemic or iontophoretic administration of D_2 receptor agonists markedly depresses the firing frequency of dopamine neurons (Wang, 1981; White and Wang, 1984). Likewise, D_2 receptor antagonists increase dopamine cell firing frequency, indicating that tonic inhibitory tone is being supplied by somatodendritically released dopamine (Wang, 1981). It would be interesting to determine the role for somatodendritically released dopamine produced by ethanol in regulating neurotransmission in the mesolimbic dopamine pathway. Further experiments will be necessary to describe the effects of focal application of ethanol into the ventral tegmental area on dopamine release in the nucleus accumbens.

The present study demonstrates that perfusion with ethanol into the ventral tegmental area stimulates serotonin release. Although the exact role for elevated serotonin in the ventral tegmental area induced by ethanol observed in this study is unknown, a number of studies have shown the existence of a functional relationship between serotonin and dopamine neurons in the ventral tegmental area. Redgrave and Horrell (1976) reported that localized perfusion of serotonin in the ventral mesencephalon potentiated medial forebrain bundle electrical self-stimulation, suggesting that serotonin may be increasing the excitability of dopamine cell bodies in the ventral tegmental area regions. Beart and McDonald (1982) showed that serotonin enhanced both spontaneous and K^+-evoked efflux of [^{3}H]dopamine in slices of the ventral tegmental area, although this result should be interpreted with caution because [^{3}H]dopamine is taken up by serotonergic terminals in this region in the absence of a serotonin uptake blocker (Chen and Reith, 1993a). Consistent with an excitatory action of serotonin on the mesoaccumbens dopamine projection, an investigation by Guan and McBride (1989) demonstrated that microinfusion of serotonin into the ventral tegmental area increases the extracellular concentration

of dopamine in the nucleus accumbens. More recently, Brodie et al. (1995) revealed that serotonin and serotonin receptor agonists potentiate the excitatory action of ethanol on ventral tegmental area dopamine neurons. Additional studies are required to clarify the role of ventral tegmental area serotonin in the reinforcing actions of ethanol.

Our data show that ethanol elicited increments in dialysate level of both dopamine and serotonin without selectivity. These results are different from those reported by Yoshimoto et al. (1991, 1992) and Heidbreder and De White (1993). They demonstrated that increases in dopamine but not in serotonin release in the nucleus accumbens were observed after ethanol 1 g/kg i.p. or 50 mM infusion into the nucleus accumbens although 2 g/kg i.p. or 100 mM perfusion caused both dopamine and serotonin to increase, suggesting a preferential sensitivity of dopamine transmission in the nucleus accumbens to ethanol. This discrepancy may be due to the different brain structure studied and ethanol's dose used. It has been demonstrated that the properties of dopamine release in the ventral tegmental area, a cell body area, might be different from the dopamine release that occurs in the nucleus accumbens, a region of dopamine nerve terminals (Kalivas and Duffy, 1991).

The results presented here show that ethanol-stimulated dopamine and serotonin releases from the ventral tegmental area were independent of the presence of Ca^{2+} in the perfusion medium. One may argue that this Ca^{2+} independence was due to incomplete elimination of Ca^{2+} from the microenvironment in which dopamine and serotonin were monitored. Indeed, in vivo studies performed with ion-selective microelectrodes have demonstrated that extracellular Ca^{2+} ions were only reduced but not completely eliminated from the microenvironment in which the dialysis perfusion medium was acutely depleted of Ca^{2+} (Benveniste et al., 1989).

In order to characterize further the mechanism by which ethanol stimulates dopamine and serotonin release, the experiments with tetrodotoxin were performed. The application of the neurotoxin tetrodotoxin is a well established technique to manipulate the electrical activity of neurons. Infusion of minor quantities of tetrodotoxin during a dialysis experiment could indicate whether neuronal activity is involved in drug-induced transmitter release. Using this method it is possible to discriminate between action potential-dependent release and action potential-independent release in the brain of freely moving animals (Westerink et al., 1987). Our data indicate that tetrodotoxin (1 μM) infusion produced dramatic reduction of dopamine and serotonin in the dialysates under basal conditions (decreased to 19.3% of baseline for dopamine and 18.3% of baseline for serotonin) but failed to block ethanol-evoked dopamine or serotonin release. Indeed, co-infusion of ethanol with tetrodotoxin caused dopamine and serotonin output to increase to the same extent as observed with Ca^{2+}-free perfusion, suggesting that, at least in part, a non-exocytotic mechanism is involved. Our findings are in agreement with other investigators who reported that the ethanol-induced increase in the frequency of miniature end-plate potentials did not require the presence of external Ca^{2+} (Quastel et al., 1971). The present results are also consonant with previous electrophysiological studies in which Brodie et al. (1990) showed that direct excitatory actions of ethanol on ventral tegmental area dopamine neurons persisted in low-Ca^{2+}, high-Mg^{2+} medium. Wozniak et al. (1991), however, demonstrated that ethanol-induced dopamine release from the striatum of anesthetized rats appeared to be Ca^{2+}-dependent. This discrepancy may be due to certain procedural differences such as the use of the ventral tegmental area vs. striatum or awake vs. anesthetized rats between this study and that reported by Wozniak et al. (1991).

It has been reported that the blood alcohol concentration in intoxicated humans is 5–50 mM (Carboni et al., 1993; Lovinger et al., 1989). An approximate 278 mM ethanol in the surrounding brain tissue was expected to be obtained when 10% (2174 mM) ethanol was administered focally assuming that in vivo probe recovery for ethanol is equal to in vitro recovery (12.8%). One may argue that such a high concentration of ethanol perhaps induces the immediate and irreversible destruction of both dopamine neuron cell bodies and serotonin terminals in the ventral tegmental area and that this underlies the observed rapid and massive increase in extracellular dopamine as well as serotonin levels. However, this possibility could be ruled out since a second perfusion with high K^+ solution into the ventral tegmental area after administration of 10% ethanol still produced an increase in dopamine and serotonin, similar in magnitude to the response to the first perfusion with 100 mM K^+ solution. This indicates that 10% ethanol did not cause irreversible damage to either dopamine cell bodies or serotonin terminals in the ventral tegmental area.

In summary, the present study represents the first demonstration that focal application of ethanol into the ventral tegmental area leads to increases in extracellular concentration of both dopamine and serotonin. These increases may reflect direct activation of dopamine neurons and serotonin terminals. Ethanol-induced dopamine and serotonin release under our experimental condition may be mediated, at least in part, via a non-exocytotic mechanism. Nonspecific stimulation of monoamine release in the ventral tegmental area by ethanol might be an important element contributing to its reinforcing properties.

References

Beart, P.M. And D. McDonald, 1982, 5-Hydroxydopamine and 5-hydroxytryptaminergic-dopaminergic interactions in the ventral tegmental area of rat brain, J. Pharm. Pharmacol. 34, 591.

Benveniste, H., A.J. Hansen and N.S. Ottosen, 1989, Determination of brain interstitial concentrations by microdialysis, J. Neurochem. 52, 1741.

Bozarth, M.A. and R.A. Wise, 1981, Heroin reward is dependent on a dopaminergic substrate, Life Sci. 29, 1881.

Brodie, M.S., S.A. Shefner and T.V. Dunwiddie, 1990, Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro, Brain Res. 508, 65.

Brodie, M.S., R.D. Trifunovic and S.A. Shefner, 1995, Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains, J. Pharmacol. Exp. Ther. 273, 1139.

Carboni, S., R. Isola, G.L. Gessa and Z.L. Rossetti, 1993, Ethanol prevents the glutamate release induced by N-methyl-D-aspartate in the rat striatum, Neurosci. Lett. 152, 133.

Chen, N.H. and M.E.A. Reith, 1993a, [^{3}DA]Dopamine and [^{3}H]serotonin release in vitro induced by electrical stimulation in A9 and A10 dopamine regions of rat brain: characterization and responsiveness to cocaine, J. Pharmacol. Exp. Ther. 267, 379.

Chen, N.H. and M.E.A. Reith, 1993b, Dopamine and serotonin release-regulating autoreceptor sensitivity in A9/A10 cell body and terminal area following withdrawal of rats from continuos infusion of cocaine, J. Pharmacol. Exp. Ther. 267, 1445.

Chen, N.H. and M.E.A. Reith, 1994a, Effects of locally applied cocaine, lidocaine, and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships, J. Neurochem. 63, 1701.

Chen, N.H. and M.E.A. Reith, 1994b, Autoregulation and monoamine interactions in the ventral tegmental area in the absence and presence of cocaine: a microdialysis study in freely moving rats, J. Pharmacol. Exp. Ther. 271, 1597.

Fouriezos, G. and R.A. Wise, 1976, Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motion or performance deficits, Brain Res. 103, 377.

Gessa, G.L., F. Muntoni, M. Collu, L. Vargiu and G. Mereu, 1985, Low doses of ethanol activate dopaminergic neurons of the ventral tegmental area, Brain Res. 248, 210.

Guan, X.M. and W.J. McBride, 1989, Serotonin microinjection into the ventral tegmental area increases accumbens dopamine release, Brain Res. Bull. 23, 541.

Gysling, K. and R.Y. Wang, 1983, Morphine-induced activation of A_{10}-dopamine neurons in the rat, Brain Res. 227, 119.

Heidbreder, C. and P. De White, 1993, Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens, Pharmacol. Biochem. Behav. 46, 477.

Herve, D., V.M. Pickle, T.H. Hoh and A. Beaudet, 1987, Serotonin axon terminals in the ventral tegmental area of the rat: Fine structure and synaptic input to dopaminergic neurons, Brain Res. 435, 71.

Hodge, C.W., M. Haraguchi, H. Erickson and H.H. Samson, 1993, Ventral tegmental microinjections of quinpirole decrease ethanol and sucrose-reinforced responding, Alcohol Clin. Exp. Res. 17, 370.

Imperato, A. and G. Di Chiara, 1986, Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol, J. Pharmacol. Exp. Ther. 239, 219.

Kalivas, P.W. And P.A. Duffy, 1991, A comparison of axonal and somatodendritic dopamine release using in vivo dialysis, J. Neurochem. 56, 961.

Klitenick, M.A., P. DeWitte and P.W. Kalivas, 1992, Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study, J. Neurosci. 12, 2623.

Lanca, A.J., 1994, Reduction of voluntary alcohol intake in the rat by modulation of the dopaminergic mesolimbic system: transplantation of ventral mesencephalic cell suspensions, Neuroscience 58, 359.

LeMoal, M., L. Stinus and H. Simon, 1979, Increased sensitivity to (+)-amphetamine self-administration by rats following mesocorticolimbic dopamine neurons destruction, Nature (London) 280, 156.

Lovinger, D.M., G. White and F.F. Weight, 1989, Ethanol inhibits NMDA-activated ion currents in hippocampal neurons, Science 243, 1721.

Lyness, W.H., N.M. Friedle and K.E. Moore, 1979, Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on *d*-amphetamine self-administration, Pharmacol. Biochem. Behav. 11, 553.

Matthews, R.T. and D.C. German, 1984, Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine, Neuroscience 11, 617.

Moore, R.Y., A.E. Halaris and B.E. Jones, 1978, Serotonin neurons of the midbrain raphe: ascending projections, J. Comp. Neurol. 180, 417.

Oades R.D., and G.M. Halliday, 1987, Ventral tegmental (A10) system: Neurobiology. 1. Anatomy and connectivity, Brain Res. Rev. 12, 117.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Pfeffer, A.O. and H.H. Samson, 1988, Haloperidol and apomorphine effects on ethanol reinforcement in free-feeding rats, Pharmacol. Biochem. Behav. 19, 343.

Portas, C.M., P. Devoto and G.L. Gessa, 1994, Effect of ethanol on extracellular 5-hydroxytryptamine output in rat frontal cortex, Eur. J. Pharmacol. 270, 123.

Quastel, M.J., J.T. Hackett and J.D. Cooke, 1971, Calcium: is it required for transmitter secretion?, Science 172, 1034.

Rassnick, N., L. Pulvirenti and G.F. Koob, 1993, SDZ-205, 152, a novel dopamine receptor agonist, reduces oral ethanol self-administration in rats, Alcohol 10, 127.

Redgrave, P. and R.I. Horrell, 1976, Potentiation of central reward by localized perfusion of acetylcholine and 5-hydroxytryptamine, Nature 262, 305.

Roberts, D.C.S. and G.F. Koob, 1983, Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats, Pharmacol. Biochem. Behav. 17, 901.

Ruggeri, M., U. Ungerstedt, L. Agnati, V. Mutt, A. Harfstrand and K. Fuxe, 1987, Effects of cholecystokinin peptides and neurotension on dopamine release and metabolism in the rostral and caudal part of the nucleus accumbens using intracerebral dialysis in the anaesthetized rat, Neurochem. Int. 10, 509.

Samson, H.H., G.A. Tolliver, M. Haraguchi and C.W. Hodge, 1992, Alcohol self-administration: role of mesolimbic dopamine, Ann. NY Acad. Sci. 654, 242.

Santiago, M. and B.H. Westerink, 1992, Simultaneous recording of the release of nigral and striatal dopamine in the awake rat, Neurochem. Int. 20, Suppl. 107s.

Seeman, P. and T. Lee, 1974, The dopamine-releasing actions of neuroleptics and ethanol, J. Pharmacol. Exp. Ther. 190, 131.

Simon, H., B. Scatton, and M. LeMoal, 1980, Dopaminergic A10 neurons are involved in cognitive functions, Nature (London) 286, 150.

Wang, R.Y., 1981, Dopaminergic neurons in the rat ventral tegmental area. II. Evidence for autoregulation, Brain Res. Rev. 3, 141.

Weiss, F., M.T. Lorang, F.E. Bloom and G.F. Koob, 1993, Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants, J. Pharmacol. Exp. Ther. 267, 250.

Weiss, F., M. Mitchiner, F.E. Bloom and G.F. Koob, 1990, Free-choice responding for ethanol versus water in alcohol-preferring (P) and unselected Wistar rats is differentially altered by naloxone, bromocriptine and methysergide, Psychopharmacology 101, 178.

Westerink, B.H.C., J. Tuntler, G. Damsma, H. Rollema and J.B. De Vries, 1987, The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis, Naunyn-Schmied. Arch. Pharmacol. 336, 502.

White, F.J. and R.Y. Wang, 1984, Pharmacological characterization of dopamine autoreceptors in the rat ventral tegmental area: microiontophoretic studies, J. Pharmacol. Exp. Ther. 231, 275.

Wise, R.A., 1978, Neuroceptic attenuation of intracranial self-stimulation reward or performance deficits?, Life Sci. 22, 535.

Wise, R.A., 1987, The role of reward pathways in the development of drug dependence, Pharmacol. Ther. 35, 227.

Wise, R.A. and M.A. Bozarth, 1987, A psychomotor stimulant theory of addiction, Psychol. Rev. 94, 469.

Wozniak, K.M., A. Pert, A. Mele and M. Linnoila, 1991, Focal application of alcohol elevates extracellular dopamine in rat brain: a microdialysis study, Brain Res. 540, 31.

Yan, Q.S., P.K. Mishra, R.L. Burger, A.F. Bettendorf, P.C. Jobe and J.W. Dailey, 1992, Evidence that carbamazepine and antiepilepsirine may produce a component of their anticonvulsant effects by activating serotonergic neurons in genetically epilepsy-prone rats, J. Pharmacol. Exp. Ther. 261, 652.

Yoshimoto, K., W.J. McBride, L. Lumeng and T.K. Li, 1991, Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens, Alcohol 9, 17.

Yoshimoto K, W.J. Mcbride, L. Lumeng and T.K. Li, 1992, Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats, Alcoholism Clin. Exp. Res. 16, 781.