Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 477 (2003) 37–44
www.elsevier.com/locate/ejphar

Involvement of non-exocytotic mechanisms in ethanol-induced in vivo dopamine release: comparisons with cocaine

Qing-Shan Yan*

Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656, USA

Received 28 April 2003; received in revised form 28 July 2003; accepted 5 August 2003

Abstract

In order to determine whether a non-exocytotic mechanism was involved in ethanol-induced in vivo dopamine release in the nucleus accumbens, extracellular dopamine concentrations were measured via intracerebral microdialysis in freely moving Sprague–Dawley rats. Effects of ethanol on dopamine release in the nucleus accumbens were compared with those by cocaine, a drug that increases synaptic dopamine by a mechanism, which depends on neuronal activity and involves an exocytotic process. Administration of ethanol (80 mM) or cocaine (10 μM) via a dialysis probe increased extracellular dopamine concentrations in the nucleus accumbens. Pretreatments with tetrodotoxin (2 μM) or Ca^{2+} withdrawal did not block the ability of ethanol to increase nucleus accumbens dopamine. The blockade of dopamine autoreceptors by local infusion of sulpiride did not significantly alter the effect of ethanol on nucleus accumbens dopamine either. As opposed to ethanol, however, cocaine-induced increases in nucleus accumbens dopamine were sensitive to tetrodotoxin or Ca^{2+} omission. In addition, pretreatments with sulpiride significantly potentiated the effect of cocaine on extracellular dopamine concentrations. These differences in responses to tetrodotoxin, Ca^{2+} withdrawal and inhibition of dopamine autoreceptors suggest that a non-exocytotic mechanism may be involved in dopamine release in the nucleus accumbens evoked by focally applied ethanol.

Keywords: Ethanol; Cocaine; Dopamine release; Nucleus accumben; Non-exocytotic; Microdialysis

1. Introduction

Several lines of evidence suggest that mesolimbic dopamine neurons, originating in the A10 region of the ventral tegmental area, play an important role in the mediation of cognitive, rewarding, and affective functions (Blackburn et al., 1992; Koob, 1992; Koob et al., 1998; Le Moal and Simon, 1991). The ventral tegmental area dopamine neurons project mainly to the limbic system including nucleus accumbens (Oades and Halliday, 1987). A number of neurochemical studies have shown that systemic (Piepponen et al., 2002; Yan, 1999; Yim and Gonzales, 2000; Yim et al., 1998), local (Wozniak et al., 1991; Yoshimoto et al., 1992) and oral self-administrations (Gonzales and Weiss, 1998; Melendez et al., 2002; Weiss et al., 1993) of ethanol all increase extracellular dopamine concentrations in the nucleus accumbens, a terminal area of the mesolimbic dopamine pathway. The increase in synaptic levels of dopamine in the nucleus accumbens is thought to be an important mechanism by which ethanol produces reward (Hyman, 1996; Koob, 1992; Koob et al., 1998).

However, the mechanism by which ethanol increases dopamine release has not been studied fully. Although Wozniak et al.'s (1991) work showed that ethanol-induced dopamine release in the striatum might appear to be mediated through an exocytotic process, some in vitro and in vivo studies have demonstrated that a non-exocytotic mechanism may also be involved in the effects of alcohol on dopamine release. Thus, direct excitatory actions of ethanol on ventral tegmental area dopamine neurons have been reported to persist in low-Ca^{+}, high-Mg^{+} medium (Brodie et al., 1990). Consistent with these findings, focal ethanol-induced dopamine release in the ventral tegmental area measured by microdialysis has been found to be insensitive to either blockade of sodium channels with tetrodotoxin or withdrawal of Ca^{2+} from the perfusion medium (Yan et al., 1996). Eshleman et al. (1994) reported that ethanol at a physiologically relevant concentration (10 mM) enhanced dopamine release in a Ca^{2+}-independent

* Tel.: +1-309-671-8538; fax: +1-309-671-8403.
E-mail address: QSY@UIC.EDU (Q.-S. Yan).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.008

manner from a cell line that expressed the human dopamine transporter and lacked vesicular storage and release mechanisms, suggesting that ethanol may interact with the dopamine transporter by a mechanism similar to that of amphetamine and methamphetamine.

This study was designed to determine whether a non-exocytotic mechanism was involved in ethanol-induced dopamine release from the nucleus accumbens in vivo. To this end, extracellular dopamine concentrations detected by microdialysis in response to ethanol or cocaine administration were compared after the treatments with tetrodotoxin, Ca^{2+}-free medium or the dopamine D2 receptor antagonist sulpiride. It has previously been demonstrated that cocaine increased synaptic dopamine by a mechanism which depends on the dopaminergic neuronal impulse flow and involves an exocytotic process as cocaine-induced increases of dopamine concentrations were inhibited by tetrodotoxin or Ca^{2+} withdrawal and potentiated by co-administration of sulpiride (Carboni et al., 1989; Chen and Reith, 1994a).

Oral administration is most relevant methodology of drug delivery in alcohol research, but the amount of consumption and absorption of ethanol can be influenced by oral sensory and pharmacokinetic factors. As a result, achieved blood and brain ethanol concentrations by oral administration may vary dramatically among animals. For example, Ferraro et al. (1991) reported that ethanol concentrations in the rat striatum varied from 1.6 to 7 mM 10 min after self-administering 5% ethanol for 10 min. In addition, systemic administration of ethanol has been found to stimulate mesolimbic dopamine neurons in the ventral tegmental areas leading to increased dopamine release in the nucleus accumbens (Gessa et al., 1985). Based on these considerations, in this study, ethanol was administered via retrograde dialysis into the nucleus accumbens to circumvent the pharmacokinetic and orosensoty factors and to minimize the effects of the ethanol on non-nucleus accumbens structures that may influence accumbal dopamine release directly and/or indirectly.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 250–300 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 maintained under 12-h light/12-h dark condition with ad libitum access to food and water. All animal care and experimentation were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria.

2.2. Drugs

Dehydrated alcohol was obtained from American Reagent Laboratories, (Shirley, NY, USA) and diluted with artificial cerebrospinal fluid (ACSF) for perfusion. Tetrodotoxin and cocaine hydrochloride were purchased from Sigma (St. Louis, MO, USA). *S*(−)-sulpiride was obtained from Research Biochemicals International (RBI, Natick, MA, USA). 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, 2000). 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 right nucleus accumbens and attached to the skull with dental acrylic and machine screws. The coordinates relative to bregma were: AP + 1.7 mm, L 0.8 mm (Paxinos and Watson, 1998). The period of post-surgical recovery was at least 5 days. On the evening of the day before the experiment, each rat was placed in a plexiglas chamber and a dialysis probe (2 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 nucleus accumbens while gently restraining the freely behaving rat. ACSF, which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155), was perfused at 0.2 μl/min overnight. On the experimental day, the ACSF flow rate was increased to 1.5 μl/min. After 2–3 h, dialysate samples from the nucleus accumbens were collected at 20-min intervals by a CMA/170 refrigerated fraction collector into vials containing 5 μl 0.1 N HCl, and stored at −80 °C until analysis. Frozen samples showed no signs of degradation for up to 1 month in our previous studies (Yan, 2000; Yan and Yan, 2001). Five consecutive samples were collected for determination of basal dopamine concentrations. All treatments were administered via a dialysis probe by using CMA/110 liquid switches. In one experiment, Ca^{2+} was withdrawn from ACSF and the Ca^{2+} chelator EGTA (1 mM) was added. 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 procedures

Dialysate samples were injected onto a high performance liquid chromatography system with electrochemical detection for determination of dopamine. This system consisted of an ESA solvent delivery system (model 580), an ESA microbore column (MD-150 × 1/RP-C18, 3 μM), and an

ESA coulochem II electrochemical detector equipped with a dual electrode analytical cell (Model 5041) and a guard cell (Model 5020). The guard cell was set at 400 mV, electrode 1 at −100 mV, and electrode 2 at 175 mV with respect to palladium reference electrodes. A VICI micro-electric two-position valve actuator with a 5-μl injection loop was used for sample injection. The mobile phase contained 75 mM Na_2HPO_4, 1.53 mM sodium dodecyl sulfate, 25 μM EDTA, 100 μl/l triethylamine, 11.5% acetonitrile and 11.5% methanol (pH 5.6 with H_3PO_4), and was pumped through the system at 0.07 ml/min. Chromatograms were integrated, compared with standards run separately on each experimental day, and analyzed using a computer-based data acquisition system (EZChrom Chromatography Data System, Scientific Software, San Ramon, CA, USA). The detection limit for dopamine was 0.5–1 fmol at a 2:1 signal-to-noise ratio.

After completion of the dialysis, the animals were given an intracardiac perfusion with buffered saline and 10% formalin solutions under anesthesia with sodium pentobarbital, and then decapitated. The brains were removed quickly, and 40-μm thick coronal sections were cut on a freezing microtome, stained with neutral red and analyzed in the light microscope. The heavy staining of gliosis along the guide cannula track permitted reliable location of the deepest point of penetration. A 2-mm-long dialysis membrane extended below the tip of the guide cannula. The point of the probe tip was then marked on coronal sections from the atlas of Paxinos and Watson (1998). Data from animals where the probes were not located within the nucleus accumbens were not included in the present study.

2.5. Data analysis

All values of dopamine reported herein represent uncorrected dialysate levels, expressed as fmol/μl of dialysate, and calculated as means ± S.E.M. A one-way or two-way analysis of variance (ANOVA) with repeated measures followed by Dunnett's or Tukey's Tests were applied. All analyses were performed through computer-based software (SigmaStat). The criterion of significance was set at $P < 0.05$.

3. Results

3.1. Effects of perfusion with tetrodotoxin or the Ca^{2+}-free medium on the 80 mM ethanol-induced dopamine efflux in the nucleus accumbens (Fig. 1)

Control experiments showed that neither perfusion with ACSF alone for 6 h nor switching between syringes containing ACSF significantly altered extracellular dopamine concentrations in the nucleus accumbens (data not shown). Fig. 1A shows the effects of perfusion with 80 mM ethanol alone on the extracellular dopamine concentration in the nucleus accumbens. Prior to ethanol exposures, the basal level of dopamine was 1.90 ± 0.24 fmol/μl of dialysate ($n = 6$). Local administration of 80 mM ethanol for 2 h elicited an immediate and significant increase in the dialysate level of dopamine. Extracellular dopamine concentrations after 2 h ethanol infusion were within the range of 2.65–2.94 fmol/μl of dialysate (139–155% of baseline). Addition of tetrodotoxin (2 μM) to or omission of Ca^{2+} from the perfusion medium caused basal dopamine concentrations to decrease dramatically (Fig. 1B and C). Prior to tetrodotoxin and Ca^{2+} withdrawal, the basal dopamine values were 1.82 ± 0.20 ($n = 6$) and 2.06 ± 0.26 ($n = 5$) fmol/μl of dialysate, respectively. At 1 h after the treatments with tetrodotoxin and the Ca^{2+}-free medium, dopamine

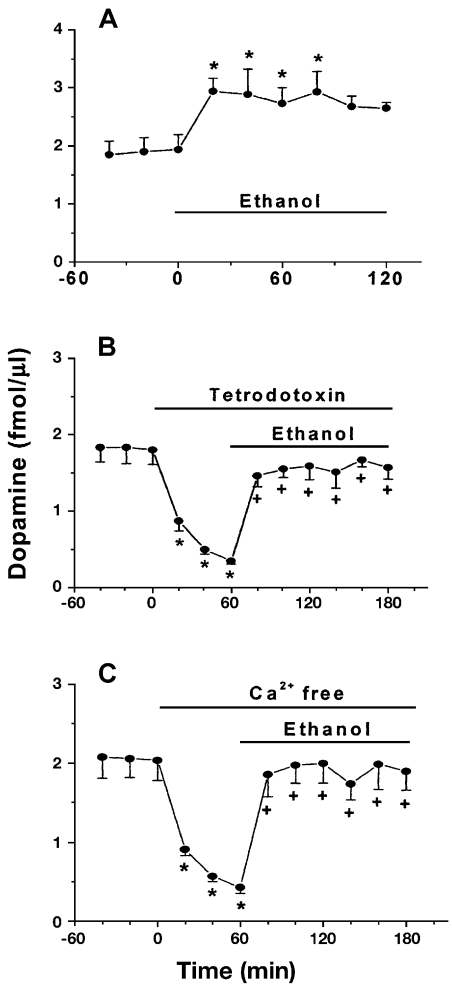

Fig. 1. Effects of tetrodotoxin or Ca^{2+} withdrawal on ethanol-induced dopamine release in the nucleus accumbens. Panel A shows the effect of perfusion with 80 mM ethanol on the extracellular dopamine concentration in the nucleus accumbens. Panels B and C show effects of tetrodotoxin and Ca^{2+} withdrawal on the effects of ethanol, respectively. Ethanol (80 mM), tetrodotoxin (2 μM), and the Ca^{2+}-free medium were administered via a dialysis probe into the nucleus accumbens indicated by the bars, respectively. Results are means with S.E.M. of data obtained from six (Panel A), six (Panel B), and five rats (Panel C). $^*P < 0.05$ as compared with the mean at the time = 0; $^+P < 0.05$ as compared with the mean at the time = 60 min (a one-way ANOVA with repeated measures followed by Dunnett's Test).

levels decreased to 0.35 ± 0.04 (19% of baseline, $P < 0.05$ as compared with pretreatment baseline) and 0.43 ± 0.08 fmol/μl of dialysate (23% of baseline, $P < 0.05$ as compared with pretreatment baseline), respectively. From this time point, ethanol was added to the medium. Inclusion of 80 mM ethanol in the presence of tetrodotoxin or in the absence of Ca^{2+} still significantly increased dopamine output in the dialysate. As shown in Fig. 1B and C, ethanol caused dopamine levels to increase from 0.35 to 1.46–1.67 and from 0.43 to 1.74–2.00 fmol/μl of dialysate in the presence of tetrodotoxin and in the absence of Ca^{2+}, respectively. The net increases flowing ethanol administration in the presence of tetrodotoxin or in the absence of Ca^{2+} were approximately 1 fmol/μl of dialysate. This value is close to that produced by 80 mM ethanol alone.

3.2. Effects of sulpiride on ethanol-induced dopamine release in the nucleus accumbens (Fig. 2)

In a separate experiment, sulpiride (20 μM), a dopamine D2 receptor antagonist, was administered via a dialysis probe into the nucleus accumbens for 4 h. Extracellular dopamine concentrations in the nucleus accumbens increased gradually, reached a stable level (130–170% of baseline) within 1 h after sulpiride infusion and remained that level throughout the experiment (data not shown).

The basal dopamine levels for the group of co-infusion of sulpiride and ethanol was 1.78 ± 0.08 fmol/μl of dialysate ($n = 6$) and not significantly different from that (1.90 ± 0.24) of the group of ethanol alone. As shown in Fig. 2A, infusion of 20 μM of sulpiride alone caused nucleus accumbens dopamine to increase slightly but significantly in this experiment. One hour later, ethanol (80 mM) was co-infused with sulpiride for another 2 h. In the presence of sulpiride, perfusion with ethanol caused dopamine concentrations to increase further (Fig. 2A). However, extracellular dopamine concentrations after co-administration of ethanol and sulpiride were not statistically different from those produced by infusion of ethanol alone ($P = 0.307$ for treatment, $P = 0.333$ for treatment × time interaction, a two-way ANOVA with repeated measures followed by Tukey's Test, Fig. 2B).

3.3. Effects of perfusion with tetrodotoxin or the Ca^{2+}-free medium on the cocaine-induced dopamine efflux in the nucleus accumbens (Fig. 3)

In the group of cocaine infusion alone, the basal dopamine value was 1.69 ± 0.15 fmol/μl of dialysate ($n = 6$). As shown in Fig. 3A, infusion of 10 μM cocaine into the nucleus accumbens for 1 h augmented dramatically extracellular dopamine concentrations. Dopamine levels increased rapidly and reached maximum levels of 6.59 ± 1.07 fmol/μl of dialysate at the end of cocaine infusion.

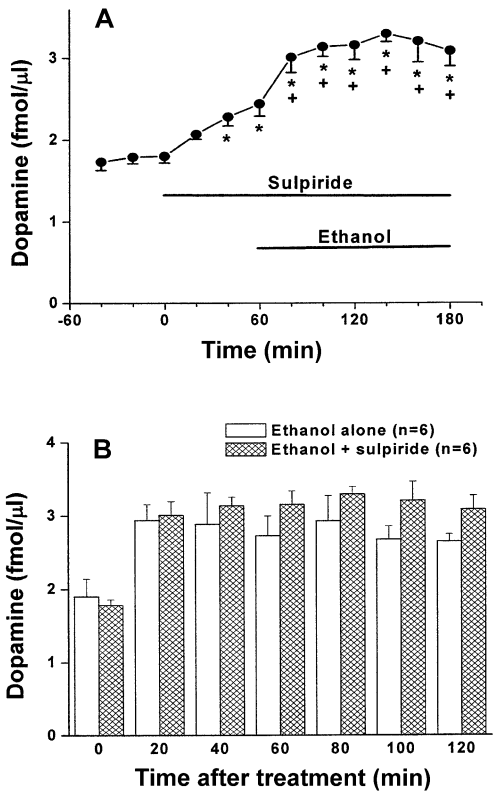

Fig. 2. Effects of sulpiride on ethanol-induced dopamine release in the nucleus accumbens. Panel A shows the time course of extracellular dopamine concentrations following sulpiride and ethanol administrations. Sulpiride (20 μM) and ethanol (80 mM) were administered via a dialysis probe into the nucleus accumbens indicated by the bars, respectively. *$P < 0.05$ as compared with the mean at the time = 0; $^{+}P < 0.05$ as compared with the mean at the time = 60 min (a one-way ANOVA with repeated measures followed by Dunnett's Test). Panel B shows comparisons of extracellular dopamine concentrations after ethanol administration in the presence and absence of sulpiride. The data of the group of ethanol alone were obtained from Panel A of Fig. 1. There were no significant differences in the means at each time point between the groups of ethanol alone and ethanol plus sulpiride (a two-way ANOVA with repeated measures).

Fig. 3B and C shows the effects of tetrodotoxin or Ca^{2+} withdrawal on the ability of cocaine to increase dopamine concentrations. In these experiments, addition of tetrodotoxin (2 μM) to or omission of Ca^{2+} from the perfusion medium caused basal dopamine concentrations to decrease dramatically (from 1.63 ± 0.18 to 0.32 ± 0.04 fmol/μl of dialysate for the tetrodotoxin group, $n = 5$, $P < 0.05$; from 2.00 ± 0.17 to 0.45 ± 0.07 fmol/μl of dialysate for the Ca^{2+}-free group, $n = 5$, $P < 0.05$). In contrast to ethanol, however, perfusion with tetrodotoxin or the Ca^{2+}-free medium all completely blocked the effects of cocaine on extracellular nucleus accumbens dopamine. Statistical analyses showed that, in the presence of tetrodotoxin or in the absence of Ca^{2+}, there were no significant differences in the extracellular dopamine concentrations before and after cocaine administration.

3.4. Effects of sulpiride on the cocaine-induced increase of dopamine in the nucleus accumbens (Fig. 4)

The basal dopamine level for the group of co-infusion of sulpiride and cocaine was 1.74 ± 0.21 fmol/μl of dialysate ($n = 6$) and not significantly different from that (1.69 ± 0.15, $P = 0.993$) of the group of cocaine alone. As shown in Fig. 4A, perfusion with 10 μM cocaine in the presence of sulpiride caused dopamine concentrations to increase dramatically. Extracellular dopamine concentrations after co-administration of cocaine and sulpiride reached the range of 7.30–14.96 fmol/μl of dialysate that was significantly greater than that (4.09–6.59 fmol/μl of dialysate, $P < 0.001$ for treatment, $P < 0.001$ for treatment × time interaction, a two-way ANOVA with repeated measures followed by Tukey's Test) produced by cocaine alone (Fig. 4B), suggesting that sulpiride potentiated the effects of cocaine on nucleus accumbens dopamine.

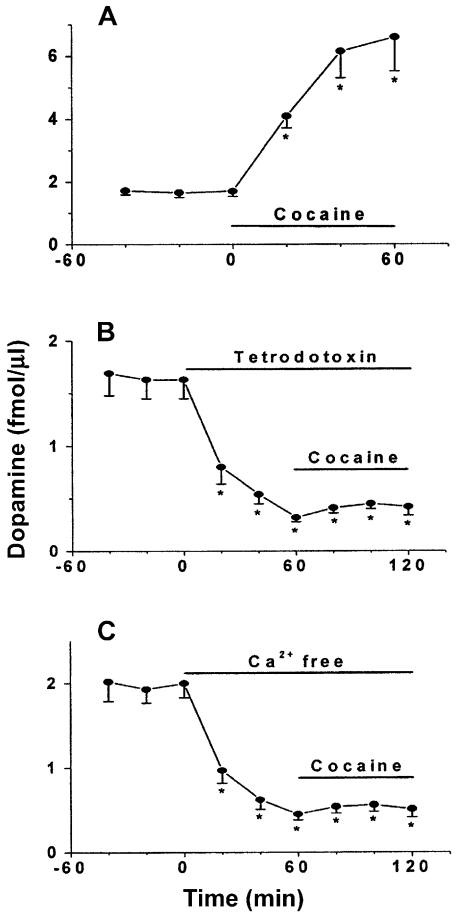

Fig. 3. Effects of perfusion with tetrodotoxin or the Ca^{2+}-free medium on the cocaine-induced dopamine efflux in the nucleus accumbens. Panel A shows the effect of perfusion with cocaine on the extracellular dopamine concentration in the nucleus accumbens. Panels B and C show effects of tetrodotoxin and Ca^{2+} withdrawal on the effects of cocaine, respectively. Cocaine (10 μM), tetrodotoxin (2 μM), and the Ca^{2+}-free medium were administered via a dialysis probe into the nucleus accumbens indicated by the bars, respectively. Results are means with S.E.M. of data obtained from six (Panel A), five (Panel B), and five rats (Panel C). $^{*}P < 0.05$ as compared with the mean at the time = 0 (a one-way ANOVA with repeated measures followed by Dunnett's Test).

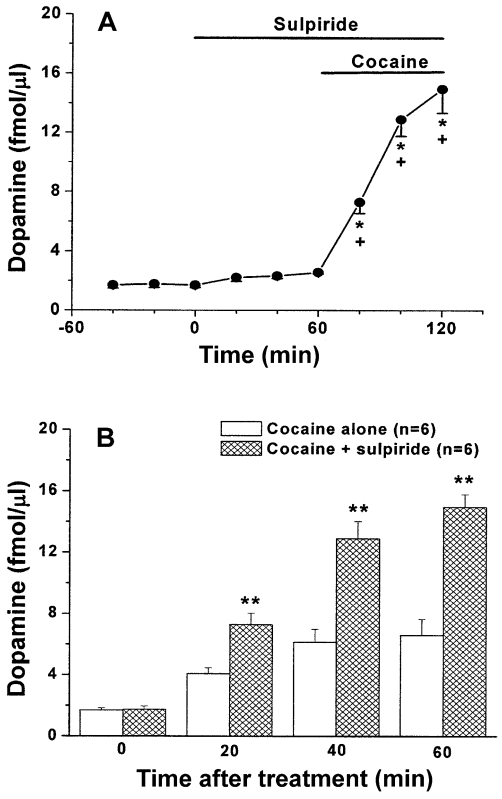

Fig. 4. Effects of sulpiride on the cocaine-induced dopamine efflux in the nucleus accumbens. Panel A shows the time course of extracellular dopamine concentrations following sulpiride and cocaine administrations. Sulpiride (20 μM) and cocaine (10 μM) were administered via a dialysis probe into the nucleus accumbens indicated by the bars, respectively. $^{*}P < 0.05$ as compared with the mean at the time = 0; $^{+}P < 0.05$ as compared with the mean at the time = 60 min (a one-way ANOVA with repeated measures followed by Dunnett's Test). Panel B shows comparisons of extracellular dopamine concentrations after cocaine administration in the presence and absence of sulpiride. The data of the group of cocaine alone were obtained from Panel A of Fig. 3. $^{**}P < 0.01$ as compared with the cocaine alone group (a two-way ANOVA with repeated measures followed by Tukey's Test).

4. Discussion

A main purpose of the present study was to investigate the mechanism by which ethanol induces dopamine release in the terminal area of the mesolimbic dopamine system. In recent years, many publications have appeared in the literature describing that, in addition to the exocytosis, neurotransmitters can exit from neurons by means of a non-exocytotic mechanism (Adam-Vizi, 1992; Levi and Raiteri, 1993). Although the role of non-exocytotic neurotransmitter release remains speculative under physiological circumstances, it is clear that some drugs of abuse like amphetamine (Kuczenski et al., 1990) and 3,4-methylenedioxymethamphetamine (MDMA, a drug of abuse termed "ecstasy") (Nash and Brodkin, 1991) exert some of their

pharmacological actions by stimulating non-exocytotic monoamine release. There is evidence that the properties of non-exocytotic release are different from those of exocytosis. For example, a non-exocytotic release, as opposed to exocytosis, is Ca^{2+}-independent, tetrodotoxin-insensitive and not regulated by presynaptic autoreceptors (Adam-Vizi, 1992; Levi and Raiteri, 1993).

Infusion of minute quantities of tetrodotoxin or the Ca^{2+}-free medium during a dialysis experiment is an established technique to indicate whether neuronal activity is involved in basal and drug-induced transmitter release (Westerink et al., 1987, 1989). In the present study, extracellular basal dopamine was inhibited by tetrodotoxin or Ca^{2+} withdrawal by 80%. The results indicate that most of extracellular dopamine in the nucleus accumbens detected by the present microdialysis method is of neuronal origin.

The present results demonstrate that local administration of 80 mM ethanol produced a significant increase in nucleus accumbens dopamine. Our data are in agreement with the previous results reported in the literature. Wozniak et al. (1991) have demonstrated that ethanol, when applied focally at the concentrations of 0.01–10% (approximately 1.7–1700 mM), produced a significant increase in dopamine in the striatum and nucleus accumbens. Yoshimoto et al. (1992) have also shown that local infusion of 50 and 100 mM ethanol into the nucleus accumbens increased extracellular dopamine to 170–200% of baseline. However, Yim et al. (1998) observed augmentations of nucleus accumbens dopamine after local infusion of ethanol only at higher concentrations (510–860 mM) but not at lower concentrations such as one used in the present study. This discrepancy may be caused by differences in the experimental conditions between the two studies. For example, ethanol was perfused for only 30 min in Yim et al.'s (1998) study but for 2 h in the present study.

An important finding of the present study is that the treatment with tetrodotoxin or Ca^{2+} withdrawal failed to block the ability of ethanol (80 mM) to increase dopamine release. Indeed, perfusion of ethanol in the presence of tetrodotoxin or in the absence of Ca^{2+} still caused extracellular dopamine concentrations to increase from 0.35 to 1.46–1.67 and from 0.43 to 1.74–2.00 fmol/μl of dialysate, respectively, suggesting that ethanol-induced dopamine release is independent of action potential propagation or the Ca^{2+} influx. On the contrary, however, the cocaine-evoked increase in extracellular dopamine concentrations was completely blocked by either tetrodotoxin or Ca^{2+} withdrawal, consistent with the previous reports showing that cocaine increased dopamine by a mechanism which involves an exocytotic process (Carboni et al., 1989; Chen and Reith, 1994b). The present data suggest that, in contrast to cocaine, focally applied ethanol may increase dopamine release via a non-exocytotic mechanism. Previous reports support this speculation. Quastel et al. (1971) reported that the ethanol-induced increase in the frequency of miniature end-plate potentials did not require the presence of external Ca^{2+}. Also, direct excitatory actions of ethanol on ventral tegmental area dopamine neurons have been found to persist in low-Ca^{2+}, high-Mg^{2+} medium (Brodie et al., 1990). Furthermore, our previous work indicated that focal application of ethanol led to a tetrodotoxin-insensitive and Ca^{2+}-independent dopamine release in the ventral tegmental area (Yan et al., 1996). Taken together, the results are consistent with the hypothesis that a non-exocytotic mechanism may be involved in ethanol-induced dopamine release. This hypothesis seems to be inconsistent with the conclusion drawn from Wozniak et al.'s (1991) study. They showed that extracellular dopamine concentrations in the striatum after perfusion with 1–3% ethanol (approximately 170–510 mM) in the presence of Ca^{2+} were significantly higher than those in the absence of Ca^{2+}. However, the authors did not compare extracellular dopamine concentrations before and after perfusion with ethanol in the absence of Ca^{2+}. Indeed, it can be seen from Wozniak et al.'s (1991) study that focal application of ethanol did cause extracellular dopamine to increase to ~140% of baseline in the absence of Ca^{2+}. In addition, the differences in the brain regions tested (nucleus accumbens vs. striatum) and the state of experimental animals (awake vs. anesthetized) between this and Wozniak et al.'s studies may also contribute to this discrepancy.

In order to characterize further the mechanism by which ethanol stimulates dopamine release, sulpiride, a dopamine D2 receptor antagonist (Stoof and Kebabian, 1984; Tagliamonte et al., 1975), was used in this study. The present study shows that local infusion of 20 μM sulpiride caused a slight but statistically significant increase in the extracellular dopamine concentration in the nucleus accumbens. Engleman et al. (2003) also observed significant increases in accumbal dopamine concentrations after infusion of sulpiride into this region. The mechanism by which sulpiride increases dopamine release involves inhibition of presynaptic release-modulating dopamine D2 autoreceptors in the nucleus accumbens. It has been demonstrated that dopamine D2 autoreceptors are coupled to K_{ATP} channels (Hausser et al., 1991; Roeper et al., 1990; Tanaka et al., 1996). Activation of K_{ATP} channels has been reported to cause hyperpolarization of the cell membrane and to shorten the duration of action potential both leading to a reduction in the Ca^{2+} influx through voltage-dependent Ca^{2+} channels and to inhibition of exocytotic dopamine release from the dopaminergic terminals (Roeper et al., 1990). Sulpiride is able to interact with presynaptic dopamine D2 autoreceptors and, therefore, facilitates exocytotic release. This concept is supported by the fact that the increase of dopamine output in the dialysate evoked by haloperidol, another dopamine autoreceptor antagonist, was prevented in the presence of the Ca^{2+} channel blocker Mg^{2+} (Westerink et al., 1989).

The present results show that the pretreatment with sulpiride did not significantly alter the effects of ethanol

on nucleus accumbens dopamine since no significant differences in the extracellular dopamine concentration were found between the groups of ethanol alone and ethanol plus sulpiride. This finding is consistent with the view that the dopamine release in the nucleus accumbens induced by focally applied ethanol is insensitive to terminal dopamine autoreceptor regulation, a feature of a non-exocytotic process (Adam-Vizi, 1992; Levi and Raiteri, 1993).

In contrast to the interaction between sulpiride and ethanol, the pretreatment with the same concentration of sulpiride significantly enhanced the effects of cocaine on nucleus accumbens dopamine. Local infusion of cocaine elicited a more pronounced increase in extracellular dopamine concentrations in the presence of sulpiride than in the absence of that drug. Previous studies showed that combination of haloperidol and the dopamine uptake blocker GBR 12909 also induced a more pronounced increase in the dopamine release which was of a synergistic nature (Westerink et al., 1987). Interestingly, sulpiride also potentiated the effects of local and systemic cocaine on the extracellular dopamine concentration in the ventral tegmental area (Chen and Reith, 1994a). Taken together, these results indicate that the dopamine uptake blocker-induced dopamine increase is regulated by presynaptic autoreceptors. Blockade of terminal dopamine D2 autoreceptors by local infusion of their antagonists such as sulpiride can abolish autoreceptor-mediated inhibitory feedback, therefore, leading to much more accumulation of dopamine in the synaptic cleft if dopamine uptake blockers are co-administered. This difference in responses to sulpiride administration between ethanol and cocaine further supports the concept that a non-exocytotic mechanism may be involved in dopamine release induced by focally applied ethanol.

It has been reported that intraperitoneal injection of ethanol at doses of 1 to 2 g/kg resulted in peak ethanol levels of 30–40 mM in the extracellular fluid of the rat nucleus accumbens (Nurmi et al., 1994; Yoshimoto and Komura, 1993). Oral self-administration of 5% ethanol for 5 min and 10–20% of ethanol for 30 min yielded ~ 28 and 16 mM of ethanol in the striatum (Ferraro et al., 1991) and blood (Czachowski et al., 1999), respectively. In the present study, perfusion with 80 mM ethanol was associated with a sizable increase in nucleus accumbens dopamine. It should pointed out that, unlike in vitro conditions, actual drug concentrations in the synapse after retrograde dialysis are much lower than those present in the perfusion medium due to (1) the probe efficiency and (2) diffusion through the interstitial space from the probe into the synaptic site. The previous study using ^{14}C-labelled ethanol showed that the ethanol recovery of the probes used in our laboratory was 12.8% (Yan et al., 1996). Considering approximately 12% of the probe efficiency, it is estimated that the actual ethanol concentration in the extracellular fluid after infusion of 80 mM may be less than 20 mM which is within the range of concentrations produced by systemically administered ethanol as mentioned above. Therefore, the non-exocytotic mechanism may make a contribution to the increase of dopamine release observed after intraperitoneal injection or oral self-administration of ethanol (see Introduction).

Nitric oxide has been found to stimulate Ca^{2+}-independent synaptic vesicle release (Meffert et al., 1994). Ethanol has effects on nitric oxide production. For example, acute ethanol administration enhanced cytokine-stimulated nitric oxide synthesis in blood–brain barrier endothelial cells (Syapin, 1998) and increased nitric oxide levels in blood and the hypothalamic-pituitary axis (Seo and Rivier, 2003). Further investigations into the molecular mechanism underlying ethanol-induced non-exocytotic neurotransmitter release are warranted.

In conclusion, the present data indicate that local administration of either ethanol or cocaine increased dopamine release in the nucleus accumbens. However, as opposed to that of cocaine, the effect of ethanol persisted in the presence of tetrodotoxin or in the absence of Ca^{2+} influx, and was insensitive to autoreceptor regulation by sulpiride. Thus, ethanol-induced dopamine release is not consistent with the criteria of exocytotic neurotransmitter release. This suggests that a non-exocytotic mechanism may be involved in the effects of focally applied ethanol on dopamine release.

Acknowledgements

This work was supported in part by a NIAAA grant (AA 10946). The author would like to thank MS. Shue Yan for her excellent technical assistance.

References

Adam-Vizi, V., 1992. External Ca^{2+}-independent release of neurotransmitters. J. Neurochem. 58, 395–405.

Blackburn, J.R., Pfaus, J.G., Phillips, A.G., 1992. Dopamine functions in appetitive and defensive behaviours. Prog. Neurobiol. 39, 247–279.

Brodie, M.S., Shefner, S.A., Dunwiddie, T.V., 1990. Ethanol increases the firing rate of dopamine neurons of the rat. Brain Res. 508, 65–69.

Carboni, E., Imperato, A., Perezzani, L., Di Chiara, G., 1989. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28, 653–661.

Chen, N.H., Reith, M.E.A., 1994a. 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, 1597–1610.

Chen, N.H., Reith, M.E.A., 1994b. 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 interrelationship. J. Neurochem. 63, 1701–1713.

Czachowski, C.L., Samson, H.H., Denning, C.E., 1999. Blood ethanol concentrations in rats drinking sucrose/ethanol solutions. Alcohol., Clin. Exp. Res. 23, 1331–1335.

Engleman, E.A., McBride, W.J., Li, T.K., Lumeng, L., Murphy, J.M., 2003. Ethanol drinking experience attenuates (−)sulpiride-induced increases in extracellular dopamine levels in the nucleus accumbens of alcohol-preferring (P) rats. Alcohol., Clin. Exp. Res. 27, 424–431.

Eshleman, A.J., Henningsen, R.A., Neve, K.A., Janowsky, A., 1994. Re-

lease of dopamine via the human transporter. Mol. Pharmacol. 45, 312–316.
Ferraro, T.N., Carrozza, D.P., Vogel, W.H., 1991. In vivo microdialysis study of brain ethanol concentrations in rats following oral self-administration. Alcohol., Clin. Exp. Res. 15, 504–507.
Gessa, G.L., Muntoni, F., Collu, M., Vargiu, L., Mereu, G., 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. 348, 201–203.
Gonzales, R.A., Weiss, F., 1998. Supression of ethanol-reinforced behavior by natrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. J. Neurosci. 18, 10663–10671.
Hausser, M.A., de Weille, J.R., Lazdunski, M., 1991. Activation by cromakalim of pre- and postsynaptic ATP-sensitive K^+ channels in substantia nigra. Biochem. Biophys. Res. Commun. 174, 909–914.
Hyman, S.E., 1996. Addiction to cocaine and amphetamine. Neuron 16, 901–904.
Koob, G.F., 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathway. Trends Pharmacol. Sci. 13, 177–184.
Koob, G.F., Roberts, A.J., Schulteis, G., Parsons, L.H., Heyser, C.J., Hyytia, P., Merlo-Pich, E., Weiss, F., 1998. Neurocircuitry targets in ethanol reward and dependence. Alcohol., Clin. Exp. Res. 22, 3–9.
Kuczenski, R., Segal, D.S., Manley, L.D., 1990. Apomorphine does not alter amphetamine-induced dopamine release measured in striatal dialysates. J. Neurochem. 54, 1492–1499.
Le Moal, M., Simon, H., 1991. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol. Rev. 71, 155–234.
Levi, G., Raiteri, M., 1993. Carrier-mediated release of neurotransmitters. Trends Neurosci. 16, 415–419.
Meffert, M.K., Premack, B.A., Schulman, H., 1994. Nitric oxide stimulates Ca(2+)-independent synaptic vesicle release. Neuron 12, 1235–1244.
Melendez, R.I., Rodd-Henricks, Z.A., Engleman, E.A., Li, T.K., McBride, W.J., Murphy, J.M., 2002. Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. Alcohol., Clin. Exp. Res. 26, 318–325.
Nash, J.F., Brodkin, J., 1991. Microdialysis studies on 3,4-methylenedioxymethamphetamine-induced dopamine release: effect of dopamine uptake inhibitors. J. Pharmacol. Exp. Ther. 259, 820–825.
Nurmi, M., Kiianmaa, K., Sinclair, J.D., 1994. Brain ethanol in AA, ANA, and Wistar rats monitored with one-minute microdialysis. Alcohol 11, 315–321.
Oades, R.D., Halliday, G.M., 1987. Ventral tegmental (A10) system: neurobiology: 1. Anatomy and connectivity. Brain Res. Rev. 12, 117–165.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, New York.
Piepponen, T.P., Kiianmaa, K., Ahtee, L., 2002. Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats. Pharmacol. Biochem. Behav. 74, 21–30.
Quastel, D.M., Hackett, J.T., Cooke, J.D., 1971. Calcium: is it required for transmitter secretion? Science 172, 1034–1036.
Roeper, J., Hainsworth, A.H., Ashcroft, F.M., 1990. Tolubutamide reverses membrane hyperpolarizations induced by activations of D2 receptors in isolated substantia nigra neurons. Pflugers Arch. 416, 473–475.
Seo, D.O., Rivier, C., 2003. Interaction between alcohol and nitric oxide on ACTH release in the rat. Alcohol., Clin. Exp. Res. 27, 989–996.
Stoof, J.C., Kebabian, J.W., 1984. Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci. 35, 2281–2296.
Syapin, P.J., 1998. Alcohol and nitric oxide production by cells of the brain. Alcohol 16, 159–165.
Tagliamonte, A., DeMontis, G., Olianas, M., Vargiu, L., Corsini, G.U., Gessa, G.L., 1975. Selective increase of brain dopamine synthesis by sulpiride. J. Neurochem. 24, 707–710.
Tanaka, T., Yoshida, M., Yokoo, H., Mizoguchi, K., Tanaka, M., 1996. ATP-sensitive K^+ channel openers block sulpiride-induced dopamine release in the rat striatum. Eur. J. Pharmacol. 297, 35–41.
Weiss, F., Lorang, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.
Westerink, B.H.C., Tuntler, J., Damsma, G., Rollema, H., de Vries, J.B., 1987. The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 502–507.
Westerink, B.H.C., Hofsteede, R.M., Tuntler, J., de Vries, J.B., 1989. Use of calcium antagonism for the characterization of drug-evoked dopamine release from the brain of conscious rats determined by microdialysis. J. Neurochem. 52, 722–729.
Wozniak, K.M., Pert, A., Mele, A., Linnoila, M., 1991. Focal application of alcohol elevates extracellular dopamine in rat brain: a microdialysis study. Brain Res. 540, 31–40.
Yan, Q.S., 1999. Extracellular dopamine and serotonin after ethanol monitored with 5-minute microdialysis. Alcohol 19, 1–7.
Yan, Q.S., 2000. Activation of 5-HT2A/2C receptors within the nucleus accumbens increases local dopaminergic transmission. Brain Res. Bull. 51, 75–81.
Yan, Q.S., Yan, S.E., 2001. Activation of $5\text{-HT}_{1B/1D}$ receptors in the mesolimbic dopamine system increases dopamine release from the nucleus accumbens: a microdialysis study. Eur. J. Pharmacol. 418, 55–64.
Yan, Q.S., Reith, M.E.A., Jobe, P.C., Dailey, J.W., 1996. Focal ethanol elevates extracellular dopamine and serotonin in the rat ventral tegmental area. Eur. J. Pharmacol. 301, 49–57.
Yim, H.J., Gonzales, R.A., 2000. Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol 22, 107–115.
Yim, H.J., Schallert, T., Randall, P.K., Gonzales, R.A., 1998. Comparison of local and systemic ethanol effects on extracellular dopamine concentration in rat nucleus accumbens by microdialysis. Alcohol., Clin. Exp. Res. 22, 367–374.
Yoshimoto, K., Komura, S., 1993. Monitoring of ethanol levels in the rat nucleus accumbens by brain microdialysis. Alcohol Alcohol. 28, 171–174.
Yoshimoto, K., McBride, W.J., Lumeng, L., Li, T.-K., 1992. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9, 17–22.