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Focal bicuculline increases extracellular dopamine concentration in the nucleus accumbens of freely moving rats as measured by in vivo microdialysis

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Abstract

This study was designed to assess the involvement of $GABA_A$ receptors in the regulation of in vivo dopamine release in the nucleus accumbens. Extracellular dopamine in the nucleus accumbens was measured using intracerebral microdialysis coupled with a high-performance liquid chromatography with electrochemical detection (HPLC–EC) system in freely moving Sprague–Dawley rats. Bicuculline, a $GABA_A$ receptor antagonist, and muscimol, a $GABA_A$ receptor agonist, were administered via a dialysis probe into the nucleus accumbens, respectively. The results showed that perfusion with bicuculline at concentrations of 25, 50, and 100 μM elicited a significant and concentration-dependent increase in extracellular dopamine in the nucleus accumbens. Dopamine levels returned to control values within 40–60 min after the termination of bicuculline perfusion. The increased dopamine produced by perfusion with 100 μM bicuculline was sensitive to sodium channel blockade with tetrodotoxin, and antagonized by co-perfusion with muscimol (25 and 50 μM) in a concentration-related fashion. Perfusion with 25 or 50 μM muscimol alone failed to alter basal levels of dopamine. The results suggest that local application of bicuculline increases dopamine release in the nucleus accumbens via a receptor-mediated process, and are consistent with the concept that basal dopamine release in the nucleus accumbens is under tonic inhibitory control by $GABA_A$ receptors within this structure.

Keywords: Dopamine; Bicuculline; Muscimol; Nucleus accumben; Microdialysis

1. Introduction

γ-Aminobutyric acid (GABA) is known to be a major inhibitory neurotransmitter in the central nervous system. It acts upon at least two pharmacologically distinct receptor subtypes, i.e., $GABA_A$ and $GABA_B$ receptors (Matsumoto, 1989). Evidence has accumulated that GABA is involved in the regulation of central dopaminergic transmission. Most studies of GABA–dopamine interactions have focused on the prefrontal cortex (Santiago et al., 1993; Grobin and Deutch, 1998) and on striatum and substantia nigra which are connected by the striatonigral GABAergic and nigrostriatal dopaminergic pathways (Santiago and Westerink, 1992; Smolders et al., 1995; Harsing and Zigmond, 1996). Recently, attention has also been paid to interactions between GABA and dopamine in the mesolimbic dopamine system.

The mesolimbic dopamine pathway, which originates in the ventral tegmental area and projects heavily to the nucleus accumbens, has been strongly implicated in reward-related processes (Le Moal and Simon, 1991; Koob, 1992; Bardo, 1998). The increase in synaptic levels of dopamine in the nucleus accumbens is thought to be an important mechanism by which drugs of abuse produce reward (Hyman, 1996; Koob et al., 1998).

Behavioral, biochemical and electrophysiological studies have demonstrated that GABA is involved in the regulation of dopaminergic transmission in the mesolimbic dopamine pathway. Microinjection of the $GABA_A$ receptor agonist muscimol into the ventral tegmental area produced a dose-dependent increase in motor activity. This effect was antagonized by either intra-tegmental injection of the $GABA_A$ receptor antagonist bicuculline or peripheral administration of haloperidol and was associated with an increase in extracellular levels of dopamine metabolites in the nucleus accumbens (Kalivas et al., 1990). Systemic administration of $GABA_A$ receptor agonists was also found

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to increase ventral tegmental area dopamine neuronal firing (Waszczak and Walters, 1980) and dopamine release in the nucleus accumbens detected by fast-cyclic voltammetry (Xi and Stein, 1998). This excitatory effect of muscimol on mesolimbic dopaminergic transmission has been postulated to be a result of indirect disinhibition of ventral tegmental area dopamine neurons. In contrast to this disinhibitory effect, intracellular recordings demonstrated that $GABA_A$ receptor agonists may directly hyperpolarize ventral tegmental area dopamine neurons, suggesting that activation of $GABA_A$ receptors located on ventral tegmental area dopamine neurons may also directly inhibit these neurons (Johnson and North, 1992; Chaudieu et al., 1994). Using a dual-probe microdialysis technique, Yoshida et al. (1993) and Westerink et al. (1996) reported that local infusion of the $GABA_B$ receptor agonist baclofen into the ventral tegmental area produced decreases in extracellular dopamine not only in the ventral tegmental area but also in the ipsilateral nucleus accumbens. Moreover, infusion of bicuculline or picrotoxin, another $GABA_A$ receptor antagonist, into the ventral tegmental area has been found to increase extracellular levels of dopamine in the ipsilateral nucleus accumbens (Westerink et al., 1996; Ikemoto et al., 1997).

The data cited above suggest that activation or blockade of GABA receptors within the ventral tegmental area can modulate dopamine release in the nucleus accumbens, a terminal area of the mesolimbic dopamine pathway. However, the significance of GABA receptors within the nucleus accumbens in the regulation of accumbal dopamine release remains largely unexplored, despite increasing interest in interactions between these two neurotransmitters and nucleus accumbens functions.

Previous studies showed that GABA neurons projecting the ventral tegmental area are located in the nucleus accumbens and ventral pallidum. Using combined retrograde labeling and in situ hybridization for glutamic acid decarboxylase mRNA, it was shown that at least 35%–55% of the neurons projecting from these structures to the ventral tegmental area are GABAergic (Kalivas, 1993). The projection is topographically organized, with the shell of the nucleus accumbens projecting to the ventral tegmental area and the accumbal core projecting to the substantia nigra (Zahm, 1989; Heimer et al., 1991). Descending GABAergic efferents to the ventral tegmental area and substantia nigra synapse on both dopaminergic and non-dopaminergic neurons (Van den Pol et al., 1985; Sugita et al., 1992). These studies provide anatomical evidence for potential local interactions between GABA and dopamine in the nucleus accumbens.

The aim of the present investigation was to study the interaction between GABA and dopamine in the nucleus accumbens via $GABA_A$ receptors. To this end, we determined if $GABA_A$ receptors within the nucleus accumbens could modulate the activity of dopaminergic transmission in this region. Thus, the effects of local perfusion with the $GABA_A$ receptor antagonist bicuculline on dopamine release in this brain area and the effects of the $GABA_A$ receptor agonist muscimol and of sodium channel blockade with tetrodotoxin on bicuculline-induced dopamine accumulation were investigated, respectively. Intracerebral microdialysis was used in this study since this technique affords a useful approach to study neurotransmitter interactions at the level of the nerve terminal in freely moving animals. All compounds tested were applied directly into the nucleus accumbens via a dialysis probe to circumvent pharmacokinetic factors and to minimize the effects of the drugs on non-nucleus accumbens structures.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 250–330 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 conditions 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

(-)-Bicuculline methbromide and muscimol hydrobromide were obtained from Research Biochemicals International (RBI, Natick, MA, USA). The compounds were dissolved with artificial cerebrospinal fluid (ACSF) to desired concentrations and protected from exposure to light during their perfusion. Bicuculline solutions were freshly prepared just before use. Tetrodotoxin was purchased from Sigma (St. Louis, MO, USA) and dissolved with ACSF to 1 μM for perfusion. 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, the rats were placed in 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 1.0 mm (Paxinos and Watson, 1986). 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 plexiglass chamber and a loop dialysis probe (2 mm in length), made from cellulose acetate hollow fibers (i.d. 215 ± 15 μm, molecular weight cut off = 6000; Spectrum Medical Industries, Los Angeles, CA, USA), was inserted into the guide and directed to the nucleus accumbens with the tip 8.2 mm below the skull surface while gently restraining the otherwise freely moving 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. The rats were directly connected to high-performance liquid chromatography (HPLC) equipment. Two fused silica tubes (i.d. 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 a VICI micro-electric two-position valve actuator (Valco Instruments, 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 (5 μl) was filled with dialysate. The valve was then switched automatically to the injection position for 20 s. This procedure was repeated every 20 min, which was the time needed to record a complete chromatogram. No treatments were administered until the basal release of dopamine was stable. All treatments were administered via a dialysis probe. Drug delivery and sample collection time were corrected for the lag time resulting from the dead volume of the inlet and outlet tubes.

Before the microdialysis experiments, all probes were tested for in vitro recovery of dopamine. Only the probes with a relative recovery of dopamine within the range of 9%–13% (the mean dopamine recovery of our probes) were used in this study to ensure that the probe performance was approximately the same.

2.4. Analytical and histological procedure

Dialysate samples were injected onto a high-performance liquid chromatography (HPLC) system with electrochemical detection. 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, 1.5 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 1 fmol at a 2:1 signal-to-noise ratio.

After completion of 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 under a light microscope. The locations of the dialysis probes were verified in each brain.

2.5. Data analysis

All values of dopamine reported herein represent uncorrected dialysate levels, expressed as fmol/fraction of dialysate, and calculated as means ± S.E.M. A one-way analysis of variance (ANOVA) for repeated measures followed by Dunnett's or Newman–Keuls test was applied.

3. Results

3.1. Effects of local infusion of bicuculline on extracellular concentrations of dopamine in the nucleus accumbens

In this experiment, three concentrations of bicuculline (25, 50, and 100 μM) were delivered into the nucleus accumbens of three groups of rats via reverse microdialysis for 80 min, respectively. The concentrations of bicuculline chosen were based on previous microdialysis studies carried out in the striatum by Smolders et al. (1995), who reported that dopamine levels in the striatum increased to a maximum of 219% of baseline following local infusion of 100 μM bicuculline. The basal extracellular concentrations of dopamine were (fmol/fraction of dialysates, mean ± S.E.M., the same below) 8.83 ± 0.59 (the 25 μM group, $n = 6$), 8.92 ± 0.92 (the 50 μM group, $n = 6$) and 8.28 ± 0.82 (the 100 μM group, $n = 6$), and did not differ significantly among the groups ($F = 0.47$, $P = 0.64$, one-way ANOVA). As shown in Fig. 1, perfusion with bicuculline at the concentrations of 25–100 μM elicited significant increases in extracellular dopamine in a concentration-dependent manner. The maximum increases of dopamine produced by perfusion with 25, 50, and 100 μM were 167%, 210%, and 314% of baseline, respectively. The dopamine concentrations in dialysates following 100 μM were higher than those following 25 μM at the time points of 40, 60, and 80 min after bicuculline administration, respectively ($P < 0.05$). Extracellular dopamine levels fell and reached the pre-treatment basal values within 40–60 min following the discontinuation of bicuculline perfusion.

In a separate control group ($n = 4$), ACSF was perfused alone into the nucleus accumbens for 6 h. No statistically significant changes in extracellular dopamine levels were

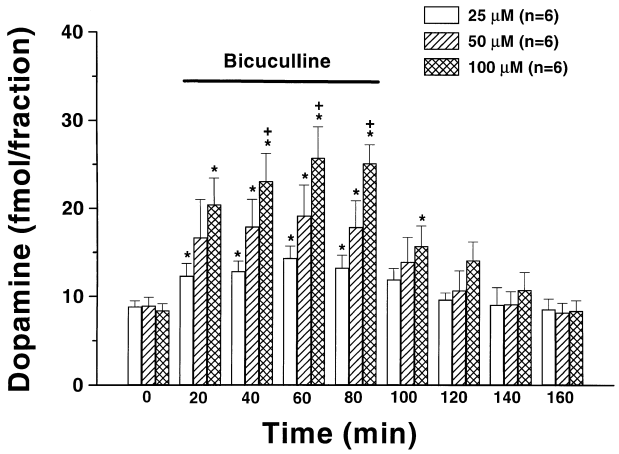

Fig 1. Effect of local infusion of bicuculline on dopamine release in the nucleus accumbens. Bicuculline (25, 50, and 100 μM in ACSF) was administered via the dialysis probe into the nucleus accumbens during the period indicated by the bar. Results are means with S.E.M. $^{*}P < 0.05$ as compared with the mean at the time = 0 for each concentration (one-way ANOVA followed by Dunnett's test). $^{+}P < 0.05$ as compared with the 25 μM groups at the corresponding time point (one-way ANOVA followed by Newman–Keuls test).

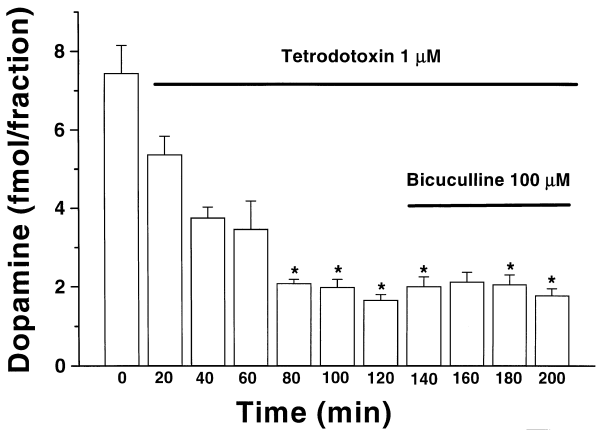

Fig 2. Effect of perfusion with tetrodotoxin on bicuculline-induced dopamine release in the nucleus accumbens. Tetrodotoxin (1 μM) and bicuculline (100 μM) were administered via the dialysis probe as indicated by the bars, respectively. Results are means with S.E.M. ($n = 5$). $^{*}P < 0.05$ as compared with the mean at the time = 0 (one-way ANOVA followed by Dunnett's test).

found during the period of 6-h ACSF perfusion (data not shown).

3.2. Effects of perfusion with tetrodotoxin on bicuculline (100 μm)-induced dopamine accumulation in the nucleus accumbens

In this experiment, tetrodotoxin (1 μM) was infused into the nucleus accumbens via a dialysis probe for 2 h and then co-infused with bicuculline (100 μM) for another 80 min. Pre-perfusion with tetrodotoxin for 2 h would allow the dialyzed tissue to be equilibrated with the toxin. Prior to tetrodotoxin treatment, the basal levels of dopamine in these animals were 7.45 ± 0.70 ($n = 5$). As can be seen from Fig. 2, perfusion with tetrodotoxin caused extracellular dopamine concentrations in the nucleus accumbens to decrease dramatically. Within 40 min following tetrodotoxin, dopamine concentrations in dialysates decreased by 50%. By 2 h, a maximal reduction of 78% was observed. From this time point, bicuculline was co-infused with tetrodotoxin for 80 min. As indicated in Fig. 2, tetrodotoxin blocked the ability of bicuculline to increase extracellular dopamine.

3.3. Effects of pretreatment with the $GABA_A$ receptor agonist muscimol on bicuculline-induced dopamine accumulation in the nucleus accumbens

In order to make a comparison with the muscimol group, 100 μM bicuculline was perfused alone into the nucleus accumbens of a separate group of rats for 80 min. The basal dopamine level in this group was 8.96 ± 1.31 ($n = 5$). In another two groups of animals, muscimol (25 and 50 μM) was infused into the nucleus accumbens for 1 h, and then co-infused with 100 μM bicuculline for another 80 min, respectively. Prior to the treatment with muscimol, the basal dopamine values in these two groups were 9.84 ± 1.21 (the 25 μM muscimol group, $n = 6$) and 7.70 ± 0.83 (the 50 μM muscimol group, $n = 7$), and were not significantly different from that of the group infused with bicuculline 100 μM alone ($F = 1.01$, $P = 0.402$, one-way ANOVA). As shown in Fig. 3, pretreatment with muscimol antagonized the effects of bicuculline on extracellular dopamine in a concentration-related manner. We can see that the higher concentrations of muscimol pro-

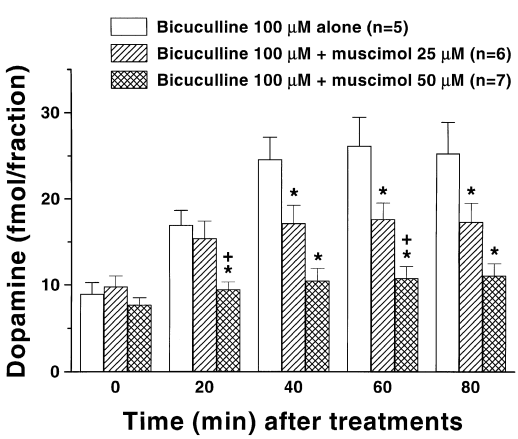

Fig 3. Effects of pretreatment with muscimol on bicuculline-induced dopamine release in the nucleus accumbens. Bicuculline (100 μM) alone was infused into the nucleus accumbens of one group of rats for 80 min. In the other two groups, muscimol (25 and 50 μM) was infused into the nucleus accumbens for 1 h, and then co-infused with bicuculline (100 μM) for another 80 min, respectively. Bicuculline (100 μM)-induced dopamine outputs were compared in the absence and presence of muscimol. Results are means with S.E.M. $^{*}P < 0.05$ as compared with the bicuculline alone group, $^{+}P < 0.05$ as compared with the 25 μM muscimol group (one-way ANOVA followed by Newman–Keuls test).

duced more pronounced inhibition of bicuculline-induced dopamine release at the time points of both 20 and 60 min after co-infusion of muscimol and bicuculline. However, perfusion with muscimol at the concentration of either 25 or 50 μM alone for 200 min did not significantly alter extracellular dopamine levels in other animals ($n = 4$ for each group, data not shown).

4. Discussion

The sodium channel blocker, tetrodotoxin, administered via the dialysis probe, has been used to determine the extent to which neurotransmitters collected in dialysate are dependent on action potential discharge (Westerink et al., 1987). In the present study, addition of tetrodotoxin (1 μm) to the perfusion medium decreased extracellular dopamine concentrations by 78%, suggesting that most extracellular dopamine in the nucleus accumbens detected by the present microdialysis method was released as a result of neuronal activity.

Previous studies showed the existence of interactions between dopamine and GABA within the nucleus accumbens. Changes in dopaminergic transmission in the nucleus accumbens produced by several drugs have been reported to be associated with drug-induced alterations in GABAergic transmission in this region. For example, the vigilance promoting drug modafinil has been found to increase dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism (Ferraro et al., 1996). Tanganelli et al. (1994) reported that the inhibition of dopamine release from the nucleus accumbens induced by neurotensin was mediated by increased GABA release in this region. A recent microdialysis study by Murai et al. (1998) showed that the α_2-adrenoceptor agonist clonidine directly or indirectly enhanced the release of GABA in the nucleus accumbens which, in turn, may decrease the release of accumbal dopamine. The present experiments confirm dopamine/GABA interactions and indicate that local infusion of bicuculline, a $GABA_A$ receptor antagonist, at the concentrations of 25–100 μM produced an augmentation of extracellular levels of dopamine in the nucleus accumbens in a concentration-dependent manner. Our results are in good agreement with those of previous studies by Ferraro et al. (1996) who observed the same effect with 50 μM bicuculline in an in vivo experiment carried out in the same brain area. They reported an increase of approximately 30% in dopamine release in response to bicuculline perfusion. This is lower than the increase (110%) seen in our experiments following perfusion with the same concentration of the drug. This difference in the magnitude of responses may be caused by different experimental conditions. For example, in the present study, rats were freely moving rather than anesthetized as in the study by Ferraro et al. (1996). It has been reported that anesthesia may suppress neurotransmitter release (Hamilton et al., 1992). Our results are also generally consistent with those of other studies reported in the literature showing that local infusion of picrotoxin (20–200 μM), another $GABA_A$ receptor antagonist, increased extracellular levels of dopamine although the studies were performed with the prefrontal cortex (Santiago et al., 1993) or ventral pallidum (Gong et al., 1998).

The present results demonstrate that when bicuculline was co-administered with locally applied muscimol, a $GABA_A$ receptor agonist, the bicuculline-induced increase of extracellular dopamine levels in the nucleus accumbens was attenuated by muscimol in a concentration-related fashion, suggesting that the effects of bicuculline on accumbal dopamine levels were mediated through $GABA_A$ receptors. Our data show that extracellular dopamine levels increased immediately upon bicuculline infusion and declined rapidly following the discontinuation of bicuculline infusion. This reversible effect of bicuculline on nucleus accumbens dopamine levels further supports the notion that bicuculline enhances extracellular dopamine levels in the nucleus accumbens via a receptor-mediated process. The finding that administration of a $GABA_A$ receptor antagonist enhanced extracellular dopamine levels in the nucleus accumbens under our experimental conditions suggests that basal dopamine release in this brain region may be under tonic inhibitory control by endogenously released GABA through activation of $GABA_A$ receptors.

Our data also show that muscimol, when administered alone at the concentrations which were effective in antagonizing the effects of bicuculline, did not alter basal levels of dopamine in the nucleus accumbens. This finding suggests that, under our experimental conditions, the $GABA_A$ receptors within the nucleus accumbens may be activated to a certain extent by endogenously released GABA so that exogenously applied muscimol cannot produce further activation at these receptors. In line with our findings, Santiago et al. (1993) reported that no effects were found on extracellular levels of dopamine in the prefrontal cortex even though the perfusion concentration of muscimol was increased to 500 μM. The present results, however, are inconsistent with those of other studies carried out in different laboratories. Ferraro et al. (1996) reported that local infusion of 50 μM muscimol into the nucleus accumbens caused extracellular dopamine levels to decrease significantly by 18%. In apparent conflict with the study by Ferraro et al. (1996), an opposite effect of muscimol on accumbal dopamine levels was observed by Yoshida et al. (1997), who showed that infusion of the same concentration (50 μM) of muscimol into the nucleus accumbens produced a delayed increase in extracellular dopamine levels in this region. In the study by Yoshida et al. (1997), the significant increase (approximately by 25%) in dopamine outflow was seen at 20–180 min following the end of drug infusion. These differences in the effects of muscimol are difficult to explain at present. In view of the fact that the sample size ($n = 4$) in the present study may

have been too small to detect relatively small differences that actually exist, further experimentation with larger samples and/or additional $GABA_A$ receptor agonists is needed to verify the effects of $GABA_A$ receptor agonists on basal dopamine release in the nucleus accumbens.

In order to evaluate the role of nerve impulses on the action of bicuculline on dopamine outflow from the nucleus accumbens, the bicuculline-induced increase in dopamine efflux was tested in the presence of tetrodotoxin (1 μM). As shown in this study, the addition of the toxin to the perfusion medium completely counteracted the facilitatory effect of bicuculline on dopamine release, suggesting a neuronal and vesicular origin of the bicuculline-evoked release of dopamine. Bicuculline could increase nucleus accumbens dopamine release by direct pre-synaptic modulation at the nucleus accumbens dopaminergic terminal. The study by Tanganelli et al. (1994) has demonstrated the presence of a local ($GABA_A$-mediated) tonic inhibitory control by GABA neurons of dopamine nerve terminals in the nucleus accumbens. It has also been reported that GABA is localized to two morphologically distinct types of neuron in the nucleus accumbens, one or both of which receive monosynaptic input from catecholaminergic afferents, and that GABAergic terminals form symmetric synapses on other principally non-GABAergic neurons (Pickel et al., 1988). This anatomical evidence suggests that indirect mechanisms may also be involved in the effects of bicuculline. This mechanism involves effects of local circuitry (e.g., GABA interneurons) and/or feedback loops (e.g., GABAergic projection neurons). The relevant circuitry may include projections to/from the ventral tegmental area. Our results showing that tetrodotoxin blocked the ability of bicuculline to increase dopamine levels are consistent with the concept that bicuculline may modulate accumbal dopaminergic transmission by influencing the transsynaptic regulation of ventral tegmental area dopamine neurons projecting to the nucleus accumbens. Further investigation is needed to clarify the mechanisms underlying the action of bicuculline. Previous work on GABAergic modulation of striatal dopamine release suggests that both direct and indirect mechanisms may be involved (Smolders et al., 1995).

In summary, the present study shows that local infusion of bicuculline into the nucleus accumbens produces a concentration-dependent increase in extracellular dopamine levels in this region. This increase in dopamine levels was reversible, sensitive to sodium channel blockade with tetrodotoxin, and antagonized by muscimol in a concentration-related fashion. Perfusion with muscimol alone did not alter significantly basal levels of dopamine. The results suggest that local application of bicuculline increases dopamine release in the nucleus accumbens via a receptor-mediated process, and are consistent with the concept that basal dopamine release in the nucleus accumbens is under tonic inhibitory control by $GABA_A$ receptors within this structure.

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