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Short Communication

Estradiol Attenuates the K^+-Induced Increase in Extracellular GABA in Rat Striatum

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ABSTRACT Estradiol acts rapidly and directly to inhibit L-type Ca^{2+} current in medium spiny neurons from striatum. Since medium spiny neurons contain γ-aminobutyric acid (GABA), we hypothesized that estradiol inhibition of Ca^{2+} channel current in the cell body would result in decreased GABA release. In this study, we examined the effect of estradiol on the concentration of GABA, taurine, and glutamate in dialysate from striatum. In support of our hypothesis, the K^+-evoked increase in GABA, but not taurine or glutamate, was significantly attenuated 30 min after treatment with estradiol. **Synapse 59: 122–124, 2006.**

INTRODUCTION

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter and estradiol inhibits GABA activity in the cerebellum, hippocampus, and hypothalamus (Ledoux and Woolley, 2005; Malyala et al., 2005; Smith et al., 2000). In addition, in whole-cell clamp recordings from acutely dissociated striatal neurons, estradiol rapidly induces a decrease in current flow through L-type Ca^{2+} channels on cell bodies of medium spiny neurons that are presumably GABAergic (Mermelstein et al., 1996). This occurs within seconds by acting at the external membrane surface to alter G-protein coupled signaling pathways (Mermelstein et al., 1996). Inhibition of Ca^{2+} current mediated by L-type Ca^{2+} channels on cell bodies decreases neurotransmitter release from neurons (Vigh and Lasater, 2004). We therefore hypothesized that estradiol would inhibit stimulated GABA release in striatum via inhibition of L-type Ca^{2+} channels at the cell body. As an initial test of this hypothesis, the present study was conducted to investigate the effect of acute estradiol administration on concentrations of GABA in dialysate from striatum. The effects of estradiol on the extracellular concentrations of taurine and glutamate were also examined.

Female Sprague-Dawley rats (200–225 g; Harlan, Indianapolis, IN) were ovariectomized and underwent in vivo microdialysis as previously described (Becker and Rudick, 1999). Fifteen to eighteen hours after dialysis probe insertion into brain through a chronic guide cannula, Ringer's solution was perfused at 1 μl/min, and on-line analysis of the dialysate was performed using capillary electrophoresis with laser-induced fluorescence, as described previously (Bowser and Kennedy, 2001; Presti et al., 2004). After basal levels of amino acids were established the rat received a subcutaneous injection of 5 μg estradiol benzoate (EB, $n = 6$) in 0.1 ml of peanut oil or 0.1 ml of peanut oil (vehicle, $n = 7$). Thirty minutes later additional electropherograms were collected to determine the effect of EB on basal GABA overflow. With the instrument continuously sampling dialysate, a 10-min, 75 mM K^+ stimulation was initiated by reverse dialysis. Electropherograms collected during the 10-min stimulation, and 30-min poststimulation period was used to determine effects of estradiol on stimulated GABA release. Probe placement in the striatum was confirmed by histological analysis.

Basal concentrations of GABA, taurine, and glutamate varied from individual to individual, but did not differ between the vehicle and EB groups, and were

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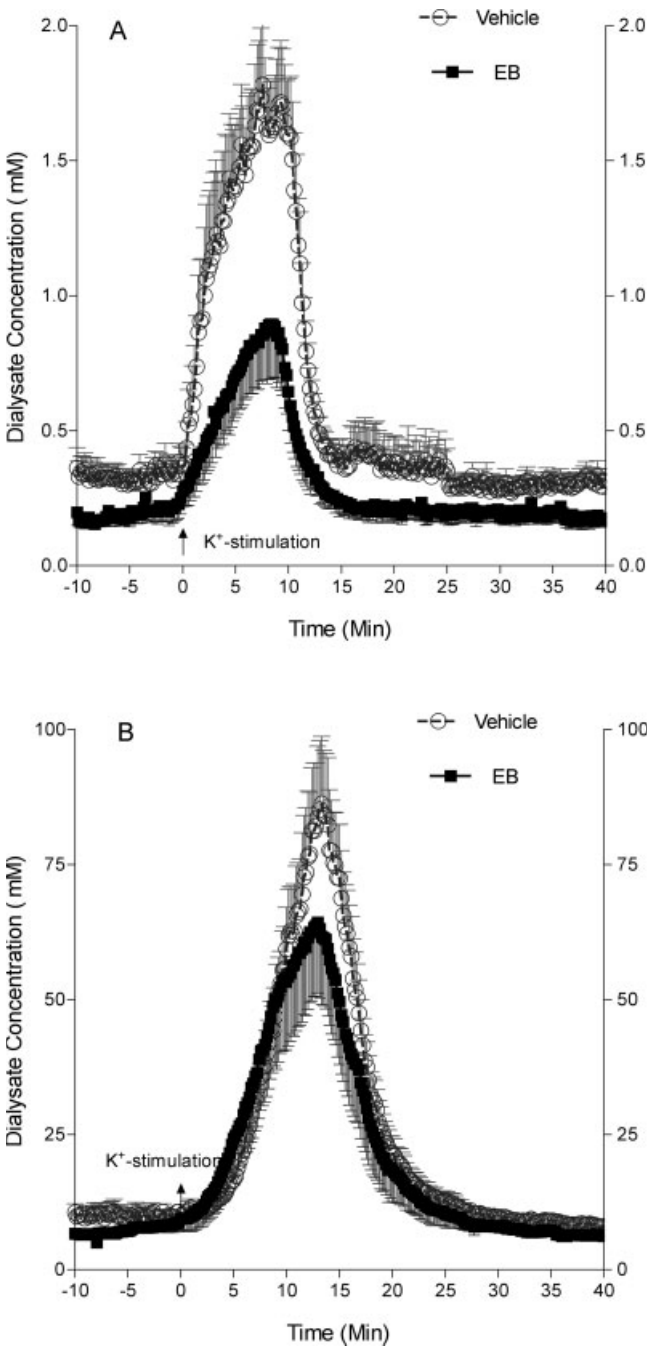

Fig. 1. Thirty minutes after injection of 5 μg of estradiol benzoate (EB) or vehicle, 75 mM K^+ was introduced by reverse dialysis for 10 min. **A**: Estradiol attenuates K^+-induced GABA in dialysate (mean ± SEM). For EB, $n = 6$; for vehicle, $n = 7$. *EB significantly inhibited K^+-induced GABA detected in dialysate relative to vehicle-treated control animals ($P < 0.028$). **B**: Estradiol does not attenuate K^+-induced taurine in dialysate (mean ± SEM). For EB, $n = 6$; for vehicle, $n = 6$ (taurine data from one animal was lost as the scale was set wrong).

not significantly affected by treatment with EB or vehicle. Local perfusion of 75 mM K^+ for 10 min produced a transient increase of GABA and taurine in the extracellular fluid from both vehicle and EB-treated rats (Fig. 1). The peak concentration of GABA was observed 8 min after the initiation of K^+ stimulation (Fig. 1A) and the peak concentration of taurine was seen 15 min after initiation of K^+ stimulation (Fig. 1B). Pretreatment with 5 μg of EB significantly attenuated the K^+-induced increase in extracellular GABA concentration in dialysate compared with that of the vehicle-treated OVX rats (Fig. 1A). Pretreatment with 5 μg of EB, however, did not significantly attenuate the K^+-induced increase in extracellular taurine concentration in dialysate compared with that of vehicle-treated OVX rats. When results were analyzed by two way ANOVA with repeated measures, there was a main effect of treatment ($F_{1,11} = 7.24$, $P < 0.021$), a treatment X time interaction ($F_{11,54} = 3.020$, $P < 0.0001$), and an effect of time of sample collection ($F_{1,54} = 23.61$, $P < 0.0001$) on increase in GABA concentration. Posthoc pairwise comparisons indicated that K^+-stimulated increase in GABA was greater for the vehicle-treated group than for the group that received EB 30 min before stimulation.

On the other hand, for taurine concentrations in dialysate there was no main effect of treatment ($F_{1,10} = 0.869$, $P = 0.37$), but there was a treatment X time interaction ($F_{10,54} = 2.94$, $P < 0.0001$), and an effect of time of sample collection ($F_{1,54} = 5.89$, $P < 0.0001$). Subsequent pairwise comparisons did not find any time points where taurine concentrations from the EB-treated rats differed significantly from the vehicle-treated OVX rats, nor was there a difference when the areas under the curve were compared.

Local perfusion of 75 mM K^+ for 10 min did not significantly affect the glutamate concentration in dialysate from either vehicle or EB-treated rats. For the vehicle group, the concentration of glutamate was 26.6 ± 4.87 pg/μl pre-K^+ and 17.8 ± 3.4 pg/μl post-K^+, while for the estradiol-treated group the concentration of glutamate was 26.2 ± 5.51 pg/μl pre-K^+ and 16.9 ± 3.5 pg/μl post-K^+.

In conclusion, our results indicate that estradiol rapidly inhibits K^+-stimulated increases in extracellular GABA in striatum, suggesting that estradiol inhibits the release of GABA from neurons. When estradiol binds to membrane-associated receptors for estradiol, L-type Ca^{2+} channels are blocked, and so the decreased Ca^{2+} is hypothesized to contribute to this reduced GABA release. Alternatively, estradiol may decrease the amount of GABA released by changing the presynaptic distribution of GABA containing vesicles as demonstrated in the hippocampus by Ledoux and Woolley (2005).

The extracellular concentrations of GABA detected using dialysis do not solely reflect exocytotic release of GABA from synapses (Del Arco et al., 2003). Nevertheless, stimulated increases in GABA detected in dialysate after methamphetamine or cocaine have been shown to reflect alterations in neural activity and function (Bustamante et al., 2002; Xi et al., 2003). Fur-

thermore, K^+-evoked increases in GABA are inhibited if tetrodotoxin is included or if Ca^{2+} is excluded from the dialysis Ringer's (Campbell et al., 1993; Hondo et al., 1995), supporting the hypothesis that such increases are derived from neuronal activity.

In this study we found that there is no effect of estradiol on the increase in taurine in dialysate following K^+ stimulation. Interestingly, the increase in K^+-stimulated taurine was delayed relative to the period of stimulation and relative to the increase in GABA. Such delays have been reported before (Ritz et al., 2002). Although the exact mechanism of K^+-evoked taurine efflux is not known, its relatively slow rise suggests its release may be secondary to other changes, or nonneuronal in origin. We also did not find an effect of estradiol on glutamate in dialysate following K^+ stimulation. From these findings we conclude that the effect of estradiol on GABA is not mediated by taurine or glutamate, and the results are consistent with a direct effect on GABA neurons.

Previous research has demonstrated that estradiol rapidly enhances stimulated dopamine (DA) release in the striatum (Becker, 1999; Becker, 2000; Xiao et al., 2003). From the effects of estradiol on stimulated GABA release, we infer that the effect of estradiol on striatal DA activity may be mediated indirectly through its effects on medium spiny GABAergic neurons. Thus, through a decrease in inhibitory tone, estradiol is hypothesized to enhance stimulated DA release, by a release of presynaptic inhibition. In support of this idea, when baclofen (a $GABA_B$ receptor agonist) is administered locally in the striatum, extracellular concentrations of DA are decreased in dialysate. Conversely, phaclofen (a $GABA_B$ receptor antagonist) administered through the dialysis probe to the striatum increased extracellular concentrations of DA in dialysate (Smolders et al., 1995). These results support the idea that $GABA_B$ receptors directly regulate DA release in striatum.

In summary, we have shown that estradiol significantly reduces K^+-stimulated increases in GABA in dialysate from female rat striatum. This finding of an inhibition of stimulated GABA supports the hypothesis that enhanced DA release evoked following estradiol treatment is mediated by $GABA_B$ receptors on DA terminals. This finding provides new insights into the mechanism(s) mediating the rapid effects of estradiol in the striatum, and is important for our understanding of how estradiol modulates neuronal activity in the striatum.

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