JPET Fast Forward. Published on March 2, 2007 as DOI: 10.1124/jpet.106.119297

1. TITLE PAGE

*Alpha*1A-Adrenergic Receptors are Functionally Expressed by a Subpopulation of CA1 Interneurons in Rat Hippocampus

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2. RUNNING TITLE PAGE

a) Running Title:

CA1 Interneurons Express α_{1A} Adrenergic Receptors

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c) Text Pages: 29

Tables: 1

Figures: 4

References: 33

Word Count for Abstract: 248

Word Count for Introduction: 458

Word Count for Discussion: 1479

JPET Fast Forward. Published on March 2, 2007 as DOI: 10.1124/jpet.106.119297
This article has not been copyedited and formatted. The final version may differ from this version.

d) Nonstandard Abbreviations:

aCSF, artificial cerebral spinal fluid

APV, D-(–)-2-amino-5-phosphonopentanoic acid

AR, adrenergic receptor

BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, tetrapotassium salt

BMY7378, 8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride

CA1, cornu ammonis 1

CNS, central nervous system

DNQX, 6,7-dinitroquinoxaline-2,3-dione

GFP, green fluorescent protein

L-765,314, (2S)-4-(4-amino-6,7-dimethoxy-2-quinazolinyl)-2-[[(1,1-dimethylethyl)amino]carbonyl]-1-piperazinecarboxylic acid, phenylmethyl ester

PE, (R)-(-)-phenylephrine hydrochloride

RT-PCR, reverse transcription-polymerase chain reaction

WB-4101, 2-[(2,6-Dimethoxyphenoxyethyl)aminomethyl]-1,4-benzodioxane hydrochloride

e) Recommended Section Assignment:

Neuropharmacology

3. ABSTRACT

The importance of adrenergic receptors (ARs) in the hippocampus has generally focused on βARs, however interest is growing in hippocampal αARs given their purported neuroprotective role. We have previously reported α_1AR transcripts in a subpopulation of CA1 interneurons. The goal of this study was to identify the specific α_1AR subtype (α_{1A}, α_{1B}, α_{1D}) functionally expressed by these cells. Using cell-attached recordings to measure action potential frequency changes, concentration response curves for the selective α_1AR agonist phenylephrine (PE) were generated in the presence of competitive subtype-selective α_1AR antagonists. Schild regression analysis was then used to estimate equilibrium dissociation constants (K_b) for each receptor antagonist in our system. The selective α_{1A}AR antagonists, 5-methylurapidil and WB-4101, produced consecutive rightward shifts in the concentration response curve for PE when used at discriminating, nanomolar concentrations. Calculated K_b values for 5-methylurapidil (10 nM) and WB-4101 (5 nM) correlate to previously published affinity values for these antagonists at the α_{1A}AR. The selective α_{1B}AR antagonist L-765,314, as well as the selective α_{1D}AR antagonist BMY7378, produced significant rightward shifts in the concentration response curve for PE only when used at non-distinguishing, micromolar concentrations. Calculated K_b values for L-765,314 (794 nM) and BMY7378 (316 nM) do not agree with affinity values for these antagonists at the α_{1B} or α_{1D}AR, respectively. Rather these K_b values more closely match equilibrium dissociation constants estimated for these compounds when used to identify α_{1A}AR subtypes. Together, our results provide strong evidence to support functional expression of α_{1A}ARs in a subpopulation of CA1 interneurons.

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4. INTRODUCTION

Adrenergic receptors (ARs) comprise a family of G protein-coupled receptors that are physiologically activated by the catecholamines epinephrine and norepinephrine. Classified into α_1, α_2, and βAR types, these receptors traditionally signal through the heterotrimeric G proteins $G_{q/11}$, G_i and G_s, respectively. The α_1AR in particular has become of increasing interest in neuroscience, as α_1AR mediated effects have been noted to regulate epileptiform activity (Weinshenker and Szot, 2002; Giorgi et al., 2004), long-term synaptic depression (Kirkwood et al., 1999; Scheiderer et al., 2004), as well as attention and cognition processes (Arnsten et al., 1999; Lapiz and Morilak, 2006). While these studies have identified α_1AR mediated effects through careful use of selective α_1, α_2, or βAR pharmacological agents, further investigation into the specific α_1AR subtypes involved has not been pursued.

Three subtypes for the α_1AR are characterized: α_{1A}, α_{1B} and α_{1D} (for review, see Graham et al., 1996). *In situ* hybridization studies in rat illustrate all three receptor subtypes are present in the central nervous system (CNS), with the α_{1A} and α_{1B}AR subtypes most prevalent (Pieribone et al., 1994; Day et al., 1997). Recent studies utilizing enhanced GFP-tagged α_{1A} or α_{1B}ARs have provided further insights into distribution of these receptors in the rodent CNS on both gross anatomical as well as cellular levels (Papay et al., 2004; 2006). Of note, these latter studies offer increased resolution of α_1AR subtype expression in the hippocampus, a cortical structure implicated in many of the neuronal processes mentioned above. α_1AR activation in the hippocampus is associated with decreased excitability of the principal pyramidal neurons (Pang and Rose, 1987; Curet and de Montigny, 1988; Mynlieff and Dunwiddie, 1988).

This effect of α_1AR activation has been attributed to increased pre-synaptic GABA release from inhibitory interneurons (Bergles et al., 1996), as well as post-synaptic NMDA receptor modulation (Scheiderer et al., 2004).

We have previously reported α_{1A}AR and α_{1B}AR mRNA present in a subpopulation of interneurons in CA1, the most apical region of the hippocampus (Hillman et al., 2005a). Cells containing these α_1AR transcripts predominate in stratum oriens and constitute a subpopulation of somatostatin-containing, GABAergic interneurons. Application of the selective αAR agonist 6-fluoronorepinephrine produces a concentration-dependent increased action potential frequency in these cells, which is blocked by the selective α_1AR antagonist prazosin but not the α_2AR selective antagonist rauwolscine (unpublished observations). While this response supports our molecular evidence of α_1AR transcription, it is unclear whether the α_{1A}, the $\alpha_{1B,}$ or both α_1AR subtypes are responsible for this increased action potential frequency. Therefore, the goal of this study was to characterize the α_1AR subtype(s) functionally expressed by this subpopulation of CA1 interneurons. Clearly deducing which α_1AR subtype excites these interneurons will provide a greater level of specificity for future studies examining the influence of AR activation in the hippocampus.

5. METHODS

Materials. APV, atropine, BAPTA, BMY7378, clonidine, DNQX, (-)-isoproterenol, L-765,314, 5-methylurapidil, MgATP, (R)-(-)-phenylephrine, NaGTP, and WB-4101 were obtained from Sigma-Aldrich (St. Louis, MO). Isoflurane was ordered from Abbott Diagnostics (Chicago, IL). All other chemical reagents were of biological grade and ordered through J.T. Baker, Inc. (Phillipsburg, NJ) or Fisher Scientific (Fairlawn, NJ).

Research Animals. Sprague-Dawley rat pups were obtained from Harlan (Indianapolis, IN) and housed with their mothers before weaning. After arrival, rats were allowed to acclimate for at least two days before their use. All protocols described have been approved by the Institutional Animal Care and Use Committee at the University of North Dakota, which is in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Slice Preparation. Sprague-Dawley rats weighing 25 to 55 g were deeply anesthetized with isoflurane and decapitated. Brains were rapidly removed and placed in chilled solution containing 110 mM choline chloride, 25 mM $NaHCO_3$, 25 mM D-glucose, 11.6 mM sodium ascorbate, 7 mM $MgSO_4$, 3.1 mM sodium pyruvate, 2.5 mM KCl, 1.25 mM $NaPO_4$, and 0.5 mM $CaCl_2$. Using an HM650V vibratome (Microm, Walldorf, Germany), 400 µm coronal sections were cut and transferred to a holding solution of 130 mM NaCl, 3.5 mM KCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 24 mM $NaHCO_3$, and 10 mM D-glucose. Slices were incubated for 30 min at 37°C and then moved to room temperature. During experimentation, individual slices were continually bathed in artificial cerebral spinal fluid (aCSF) consisting of 119 mM NaCl, 5 mM KCl,

1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 11 mM D-glucose. To pharmacologically isolate the cell of interest from primary excitatory inputs, 1 μM atropine, 10 μM DNQX, and 50 μM APV were also included in the aCSF. All solutions were continually aerated with 95% O_2, 5% CO_2.

Cell-Attached Recording. Micropipettes were prepared from borosilicate glass using a vertical PP-830 puller (Narishige, Tokyo, Japan). Pipettes were filled with an internal solution of 135 mM KCH_3SO_4, 8 mM NaCl, 10 mM HEPES, 2 mM MgATP, 0.3 mM NaGTP, and 0.1 mM $BAPTAK_4$. Using a BX51WI upright microscope (Olympus, Melville, NY), the hippocampus was visualized under infrared-differential interference contrast optics. A candidate CA1 interneuron from stratum oriens or stratum radiatum was identified and centered in the visual field. With the micropipette, a gigaohm seal was formed on the soma of the candidate interneuron, enabling an isolated recording while maintaining integrity of the cell membrane. A baseline action potential frequency was recorded for 25 min, allowing time for equilibration of an AR antagonist if warranted by the experiment. Increasing concentrations of a specified AR agonist were then added to the perfusion line in 8 min increments. Changes in action potential frequency were visualized in real time and recorded for subsequent analysis. To avoid issues of receptor desensitization and depolarization block, only one hippocampal slice was used per experimental paradigm. After a single concentration response curve was generated from an interneuron, generating an n=1, the hippocampal slice was discarded and a new slice equilibrated in aCSF. Action potentials were detected using an Axoclamp 700B (Molecular Devices Corporation, Sunnyvale, CA), digitized with a Digidata 1322A analog-to-digital converter (Molecular Devices), and recorded using Axoscope 9.2

software (Molecular Devices.). Postexperimental analysis was completed using Mini Analysis 5.0 (Synaptosoft, Decatur, GA) and Prism 4.03 (GraphPad Software Inc., San Diego, CA).

Statistical Analysis. All values are reported as the mean±S.E., $n \geq 3$ as indicated. Action potential frequencies recorded during the course of each functional experiment were used to plot a concentration response curve expressed as a percentage of the maximal AR agonist response. A fitted iterative nonlinear regression curve was used to determine the effective AR agonist concentrations that caused 50% of the maximal response (EC_{50}). The fitted iterative nonlinear regression curve that best represented the data was determined using a partial F-test, F = [(SS1–SS2)/SS2]/[(DF1–DF2)/DF2], where SS is sum of the squares and DF is degrees of freedom ($p<0.05$). Significance between experimental groups was tested using a one-way ANOVA with a post-hoc Bonferroni test (Figure 1) or an unpaired two-tailed Student's t test ($p<0.05$). Equilibrium dissociation constants (K_b) for subtype-selective α_1AR antagonists were estimated as previously described (Hillman et al., 2005b), using the method of Arunlakshana and Schild (1959). Schild regression slopes are expressed as the mean±S.E. and were considered different from unity if the 95% confidence interval did not include a value of 1 (Kenakin, 1997).

6. RESULTS

α_1AR activation produces a concentration-dependent increase in action potential frequency. In order to understand the relationship between previously characterized mRNA expression patterns and functional receptor expression, a cell-attached configuration was used to record action potential frequencies from visually identified CA1 interneurons. Interneurons that provided a stable baseline recording for at least 25 min were challenged with increasing concentrations of either the selective α_1AR agonist phenylephrine (PE), the selective α_2AR agonist clonidine, or the selective βAR agonist isoproterenol. As shown in Figure 1, PE produced a concentration-dependent increase in action potential frequencies, reaching a maximal effect at 100 μM. Contrary to the effect of PE, no change in action potential frequency was observed with increasing concentrations of clonidine or isoproterenol. Results from these initial experiments suggest a specific CA1 interneuron population is excited in response to α_1AR activation.

α_{1A}AR antagonism alters the CA1 interneuron response to PE. To determine which α_1AR subtype(s) are functionally expressed by CA1 interneurons, we examined the effect of subtype-selective α_{1A}AR antagonists on the PE-mediated increased action potential frequency. Hippocampal slices were allowed to equilibrate with fixed concentrations of competitive receptor antagonist for at least 25 min before the onset of experimentation. Pre-treatment of slices with 20 nM, 50 nM, or 100 nM of the selective α_{1A}AR antagonist 5-methylurapidil produced consecutive parallel rightward shifts in the PE concentration response curve (Figure 2A). PE dose ratios in the presence and absence of each 5-methylurapidil concentration were calculated from individual experiments and used to generate a Schild regression line (Figure 2B). This analysis estimated an

equilibrium dissociation constant (K_b) for 5-methylurapidil of 10.0±5.0 nM, a value which best correlates to previously published affinities of this competitive AR antagonist at the α_{1A}AR (Table 1).

A second subtype-selective α_{1A}AR antagonist, WB-4101, was used to further support the idea that the CA1 interneuron response to PE is mediated by α_{1A}AR activation. Fixed WB-4101 concentrations of 10 nM, 30 nM, and 50 nM produced consecutive parallel rightward shifts in the PE concentration response curve (Figure 2C). EC_{50} values obtained from these experiments were used to calculate dose ratios required for Schild regression analysis (Figure 2D), which provided an estimated K_b of 5.0±1.2 nM for WB-4101 in our system. Although WB-4101 is not as selective for the α_{1A}AR subtype as 5-methyurapidil (Table 1), the high affinity experimental value calculated for WB-4101 to inhibit PE-mediated increased action potential frequencies in CA1 interneurons suggests involvement of the α_{1A}AR and/or α_{1D}AR subtype. While α_{1A}AR expression is supported by our experimentation using 5-methyurapidil, as well as our previously published mRNA profiling, one cannot discount the possibility that α_{1D}ARs are also expressed by these cells. As evident in Table 1, the estimated K_b of WB-4101 in our system could correlate to previously published affinity values for this competitive AR antagonist at the α_{1D}AR. Therefore, we next utilized a selective α_{1D}AR competitive antagonist to test any contribution that this AR subtype may have to the PE-mediated increase in action potential frequencies observed for CA1 interneurons.

Subtype-selective α_{1D} or α_{1B}AR competitive antagonists alter CA1 interneuron responses to PE only at non-specific concentrations. The selectivity for the competitive receptor antagonist BMY7378 is over 100-fold for the α_{1D} versus the α_{1A} or α_{1B}AR

subtypes, as determined using both functional and radioligand binding techniques (Goetz et al., 1995; Piascik et al., 1995). Therefore, in order to establish if the α_{1D}AR subtype is contributing to agonist mediated changes in action potential frequency observed for CA1 interneurons, this selective α_{1D}AR antagonist was used in conjunction with increasing concentrations of PE. BMY7378 did not significantly affect the interneuron response to PE when this competitive antagonist was used at selective concentrations that block the α_{1D}AR subtype (data not shown). The lowest concentration of BMY7378 needed to provide a statistically significant shift of the PE concentration response curve was 0.5 μM (Figure 3A). PE EC_{50} dose ratios calculated from each individual experiment containing a fixed concentration of BMY7378 were subsequently used for Schild regression analysis, which estimated a K_b value of 316±120 nM for this selective α_{1D}AR antagonist in our system (Figure 3B). When compared to previous reports, this K_b value does not correlate to calculated affinities of BMY7378 for the α_{1D}AR, thus eliminating the possibility that activation of this α_1AR subtype contributes to the PE-mediated increase in CA1 interneuron action potential frequency (Table 1).

Since BMY7378 does not further discriminate between the α_{1A} or α_{1B}AR we utilized another AR competitive antagonist, L-765,314, that has demonstrated high affinity for the α_{1B}AR subtype in both functional and radioligand binding analysis (Patane et al., 1998; Jähnichen et al., 2004). L-765,314 did not produce significant shifts in the PE concentration response curve when used at nanomolar concentrations that are selective for competitively interacting with the α_{1B}AR (data not shown). Significant shifts in the PE concentration response curve were observed only when L-765,314 was used at non-selective, micromolar concentrations (Figure 4A). Schild regression plots of

the PE EC_{50} dose ratio data points versus receptor antagonist concentration estimated a 794±60 nM K_b value for L-765,314 (Figure 4B). This experimental equilibrium dissociation constant calculated for L-765,314 is approximately 150-times greater than its published binding affinity at the α_{1B}AR, which strongly suggests that this α_1AR subtype is not responsible for the PE-initiated effect observed in our system (Table 1). Furthermore, this calculated K_b is over 15-fold greater than the affinity of L-765,314 documented for the α_{1D}AR, again substantiating our results using BMY7378, which showed no involvement of the α_{1D}AR subtype in mediating this same response. Taken together, experimental K_b values obtained in our studies for both receptor antagonists L-765,314 and BMY7378 most closely associate to affinity values published for the α_{1A}AR. Therefore, the experimental K_b profile of subtype-selective α_1AR antagonists used in this study implicates functional expression of the α_{1A}AR subtype in PE-responsive CA1 interneurons.

7. DISCUSSION

The goal of this study was to functionally characterize α_1AR subtypes expressed by CA1 interneurons in rat hippocampus. Using single-cell RT-PCR we have previously shown α_1AR transcripts are present in a subpopulation of GABAergic interneurons in this region of the hippocampus (Hillman et al., 2005a). Preliminary experiments in our laboratory revealed a concentration-dependent increase of interneuron action potential frequencies following application of the selective αAR agonist 6-fluoronorepinephrine or the selective α_1AR agonist PE. While this functional response substantiated our molecular studies, it was not clear which specific α_1AR subtype(s) mediated this effect. Using four different subtype-selective α_1AR antagonists to competitively inhibit the PE-mediated increased action potential frequency, we have demonstrated here that a subpopulation of CA1 interneurons functionally express the α_{1A}AR. This report represents the first description for the functional distribution of a specific α_1AR subtype in a defined region of the hippocampus.

As summarized in Table 1, the calculated K_b profile of all four subtype-selective α_1AR antagonists used in our analysis best correlate with previously published K_i values of these same compounds for the α_{1A}AR. The competitive receptor antagonists 5-methylurapidil and WB-4101, which are most selective for the α_{1A}AR subtype, demonstrated a high affinity to inhibit PE-initiated action potentials in CA1 interneurons, suggesting α_{1A}AR subtypes are likely expressed in these cells. While our estimated K_b values for 5-methylurapidil and WB-4101 were slightly higher than earlier reported K_i values at the α_{1A}AR, this discrepancy is likely attributable to our use of hippocampal slice preparations. For example, previous studies utilizing 5-methylurapidil were performed

on transfected COS-1 (Perez et al., 1994), COS-7 (Laz et al., 1994) or fibroblast cultures (Saussy et al., 1996), allowing an isolated environment in which to study α_1AR expression. While these earlier studies provide important information regarding α_1AR subtype characterization, the goal of our investigation was to examine α_1AR subtype expression in a more physiological context within a specific region of the CNS. The use of hippocampal slices, while enabling us to identify α_1AR expression patterns of individual interneurons within the brain, also introduces intact neuronal networks not present in transfected cell systems. Although much care is taken to pharmacologically isolate the interneuron of interest from primary inputs, residual network activity likely accounts for minor discrepancies between our experimental K_b and previously published K_i values for 5-methylurapidil and WB-4101. Regardless, the high affinity values calculated for 5-methylurapidil and WB-4101 to inhibit PE-initiated action potentials in our system suggest α_{1A}ARs are likely expressed by this subpopulation of CA1 interneurons. However, we could not completely rule out the possibility that our results obtained using WB-4101 suggest expression of the α_{1A}AR and/or the α_{1D}AR subtypes.

To delineate functional expression between these two α_1AR subtypes, BMY7378, a compound which has over a 100-fold selectivity for the α_{1D} versus the α_{1A} or α_{1B}AR, was employed (Goetz et al., 1995). The calculated low affinity of BMY7378 in our system (>300 nM) suggests that α_{1D}ARs are *not* functionally responsible for the PE-mediated increase in action potential frequency seen in CA1 interneurons, substantiating our conclusions for α_{1A}AR expression based on affinity values of 5-methylurapidil and WB-4101. This calculated low affinity value for BMY7378 is supported by previous *in situ* studies which report almost no hybridization for the α_{1D}AR subtype in rat

hippocampus (Day et al., 1997), and substantiates our earlier findings that α_{1D}AR transcripts are absent in CA1 interneurons (Hillman et al., 2005a).

While the low K_b value estimated for BMY7378 in our system suggests α_{1D}ARs are not involved in the PE-mediated increase in action potential frequency, it does not further distinguish between functional expression of the α_{1A} or α_{1B}AR subtype (Table 1), therefore the selective α_{1B}AR competitive antagonist L-765,314 was utilized for further analysis. The calculated low affinity (>700 nM) of L-765,314 in our system strongly implies that the α_{1B}AR subtype does not contribute to the observed PE-mediated increased action potential frequency. L-765,314 produced significant shifts of the PE concentration response curve only when used at very high concentrations, which relates to receptor antagonism of the α_{1A} or α_{1D}AR subtype. However, the published K_i value for L-765,314 at the α_{1D}AR is 50 nM, which is 15-fold lower than the functional K_b calculated in our study for this same receptor antagonist. Consequently, this calculated equilibrium dissociation constant of L-765,314 is most indicative for the expression of an α_{1A}AR subtype, further substantiating our results using 5-methylurapidil and WB-4101. Moreover, the low affinity value calculated for BMY7378 rules out any possible contribution of an α_{1D}AR subtype mediating this PE-initiated response, significantly supporting our conclusions that only α_{1A}AR subtypes are functionally coupled to increasing action potential frequencies on these interneurons.

A lack of α_{1B}AR involvement was surprising, given the fact that transcripts for both the α_{1A} and α_{1B}AR were found in cytoplasmic samples taken from CA1 interneurons (Hillman et al., 2005a). Previous *in situ* studies also demonstrate α_{1B}AR mRNA in CA1 (Pieribone et al., 1994; Day et al., 1997), though these studies often report α_{1A}AR

expression in the hippocampus at a greater density. The predominance of α_{1A}AR subtype expression in the hippocampus is supported by binding studies (Wilson and Minneman, 1989) and more specifically in CA1 hippocampus by enhanced-GFP tagged α_1AR localization experiments (Papay et al., 2006). One could speculate that both α_{1A} and α_{1B}AR subtypes are transcribed in CA1, with only the α_{1A}AR translating into a functional protein. Considering that neurotransmitter receptor dysregulation and neuronal cell death has been observed in transgenic animals overexpressing the α_{1B}AR subtype, this lack of functional α_{1B}AR expression may be a beneficial adaptation (Yun et al., 2003).

Alternatively, the α_{1B}AR may be a functionally expressed membrane protein that simply is not linked to the receptor agonist-mediated increases in action potential frequency monitored for this study. In our experiments we elected to use single cell, cell-attached recordings to enable generation of complete concentration response curves from individual interneurons within CA1 hippocampus. While the approach allowed us to perform pharmacological analysis on single cells in an *in vitro* physiological context, this technique is limited in that it detects only the all-or-none action potential. With regard to functional α_{1B}AR or α_{1D}AR expression in CA1, monitoring subthreshold changes in membrane potential by performing whole-cell interneuron recordings may provide better insight into possible functional contributions of these other α_1AR subtypes. However, due to dialysis of cell contents over time, this more sensitive technique of whole-cell recording would not allow for generation of entire concentration response curves, thus limiting complete characterization using pharmacological analysis.

It should be noted that not all CA1 interneurons examined responded to selective α_1AR agonism. Approximately 75% of interneurons in stratum oriens and 25% in

stratum radiatum generated concentration-dependent increases in action potential frequencies following PE application. The remaining interneurons tested showed no change in baseline action potential frequency following applications of up to 100 μM PE. This is consistent with our previous molecular findings that α_1AR transcripts are present only in a subset of somatostatin positive interneurons (Hillman et al., 2005a), a subpopulation of interneurons which predominate in stratum oriens of CA1 (Freund and Buzsáki, 1996). Interestingly, voltage-activated K^+ conductance was recently shown to modulate cell excitability in this same subset of CA1 interneurons (Lawrence et al., 2006), perhaps suggesting, at least in part, a possible mechanism behind the PE-mediated interneuron depolarization we observed in this study. Presently it is important to consider our observed α_{1A}AR-mediated effects only within the confines of a specific CA1 cell population, at least until further characterizations can been made throughout the entire hippocampal network.

On a wider scope, current laboratory investigations suggest that α_{1A}AR activation on CA1 interneurons decreases activity of neighboring principal pyramidal cells via both neurotransmitter and neuropeptide release. This observation of a selective α_{1A}AR-mediated effect represents a potentially novel therapeutic target for anti-epileptic drug exploration. The hippocampus is a focal point for the majority of temporal lobe epilepsies, and CA1 neurons are particularly susceptible to cell death following instances of epileptiform activity. α_1AR activation has been shown to decrease spontaneous excitatory activity in cultured hippocampal neurons (Croce et al., 2003), as well as increase CA1 inhibitory tone in hippocampal slices (Bergles et al., 1996). In a recent case study, administration of an α_1AR antagonist in a patient with temporal lobe epilepsy

was recently shown to be highly pro-convulsant (Ivanez and Ojeda, 2006). Therefore, it can be speculated that selective activation of α_{1A}AR subtypes in the CA1 hippocampus may represent a novel way to increase inhibitory tone, which would serve to decrease epileptiform activity and promote neuronal survival in this region of the CNS.

In summary, by functionally determining affinity values for a panel of competitive subtype-selective α_1AR antagonists we demonstrate for the first time at a single cell level that α_{1A}AR activation can mediate an increased action potential frequency in CA1 interneurons. While α_{1A}AR-mediated responses cannot be assumed to be present throughout the entire hippocampus, the identification of a specific functional α_1AR subtype in CA1 will help focus future adrenergic studies in this region.

8. ACKNOWLEDGMENTS

We thank Ke Xu for assistance in preparing the manuscript.

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10. FOOTNOTES

This study was supported by a predoctoral fellowship from the American Epilepsy Society (KLH), the North Dakota Experimental Program to Stimulate Competitive Research through NSF Grant EPS-0447679 (KLH, VAD and JEP), NSF CAREER Grant 0347259 (VAD) and NIH Grant 5P20RR017699 from the Centers of Biomedical Research Excellence program (VAD and JEP). A preliminary report of these findings was presented in April 2006 at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics, and in December 2006 at the annual meeting of the American Epilepsy Society.

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JPET Fast Forward. Published on March 2, 2007 as DOI: 10.1124/jpet.106.119297
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11. LEGENDS FOR FIGURES

Figure 1. Differing effects of α_1, α_2 and βAR activation on CA1 interneuron activity. **(A)** Hippocampal CA1 interneurons were challenged with increasing concentrations of either the selective α_1AR agonist PE (□), the selective α_2AR agonist clonidine (■), or the selective βAR agonist isoproterenol (■). Only the α_1AR agonist PE produced a significant (*p<0.05, ***p<0.001) concentration-dependent increase of interneuron action potential frequency. Action potentials are normalized for each AR agonist and shown as percent of baseline; n=3. **(B)** Representative trace of action potentials recorded from a CA1 stratum oriens interneuron, demonstrating increased action potential frequency in response to α_1AR activation.

Figure 2. Effect of the selective α_{1A}AR antagonists 5-methylurapdil and WB-4101. **(A)** Pre-treatment with 20 nM (●), 50 nM (○) or 100 nM (▲) of the selective α_{1A}AR antagonist 5-methylurapidil produced parallel rightward shifts of the PE curve that were significantly different (p<0.05) from control [EC_{50} 30±4, 46±5, and 83±11 μM respectively, versus 9.4±0.5 μM control ×, n=5]. **(B)** Dose ratios calculated from each individual experiment were used for Schild regression analysis, providing an x-intercept value of -8.0±0.3 with a slope of 0.8±0.2. **(C)** Pre-treatment with 10 nM (●), 30 nM (○), or 50 nM (▲) of the selective α_{1A}AR antagonist WB-4101 produced significant (p<0.05) parallel rightward shifts of the PE curve [EC_{50} 18±3, 42±5, and 77±13 μM respectively, versus 6.4±0.4 μM control ×, n=3). **(D)** Using dose ratios calculated from each

individual experiment, a Schild plot was created generating an x-intercept value of -8.3±0.1 with a slope of 0.9±0.1.

Figure 3. Effect of the selective α_{1D}AR antagonist BMY7378. **(A)** Pre-treatment with 0.5 μM (●), 5 μM (○) or 50 μM (▲) of BMY7378 produced significant (p<0.05) parallel rightward shifts in the PE concentration response curve [EC_{50} 17±1.6 μM, 86±11 μM, 426±32 μM versus 7.7±0.15 μM control ×, n=3]. **(B)** Dose ratios calculated from each individual experiment were used for Schild regression analysis, which estimated an x-intercept value of -6.5±0.2 with a slope of 0.9±0.1.

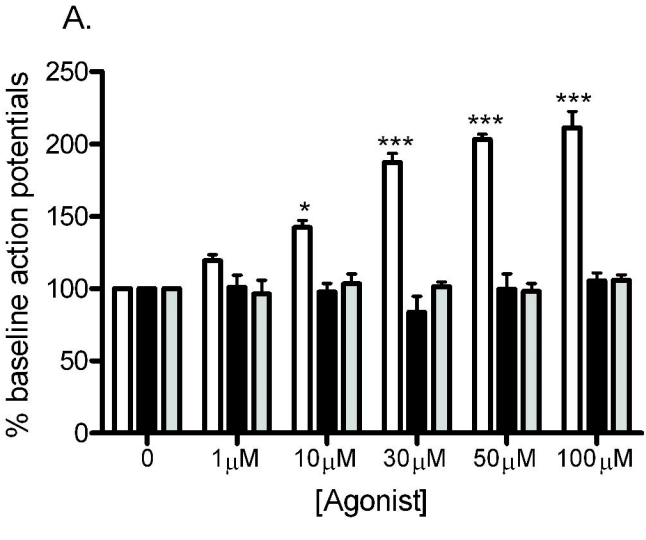
Figure 4. Effect of the selective α_{1B}AR antagonist L-765,314. **(A)** Pre-treatment with 1 μM (●), 10 μM (○) or 30 μM (▲) of L-765,314 produced significant (p<0.05) consecutive parallel rightward shifts in the PE concentration response curve for CA1 interneurons [EC_{50} 39±5 μM, 65±10 μM, and 308±68 μM respectively, versus 17±5 μM control ×, n=5]. **(B)** Dose ratios calculated from each individual were used for Schild regression analysis, which estimated an x-intercept value of -6.1±0.2 with a slope of 0.9±0.1.

12. TABLES

Table 1. Experimental K_b comparisons with published binding equilibrium dissociation constants (K_i) of subtype-selective α_1AR antagonists for recombinant α_1AR subtypes (in nM). Combined binding affinity values (mean ± S.E.) of subtype-selective AR antagonists for cloned non-human α_1AR subtypes (rat α_{1A}, α_{1B}, and α_{1D}; hamster α_{1B}) taken from Goetz et al., 1995; Laz et al., 1994; Lomasney et al., 1991; Patane et al., 1998; Perez et al., 1994; Piascik et al., 1995 and Saussy et al., 1996.

α_1AR Antagonist	Experimental K_b (*n*)	Reported K_i Values		
		α_{1A}AR	α_{1B}AR	α_{1D}AR
5-methylurapidil	10 ± 5.0 (5)	2.5 ± 1.2	274 ± 156	124 ± 79
WB-4101	5.0 ± 1.2 (3)	0.4 ± 0.3	19 ± 7	5.0 ± 3.0
BMY7378	316 ± 120 (3)	573 ± 153	466 ± 138	4.4 ± 1.3
L-765,314	794 ± 60 (5)	500 ± 20	5.4 ± 0.6	50 ± 8

FIGURE 1.


A.
% baseline action potentials
250
200
150
100
50
0
*
***
***
***
0
1μM
10μM
30μM
50μM
100μM
[Agonist]

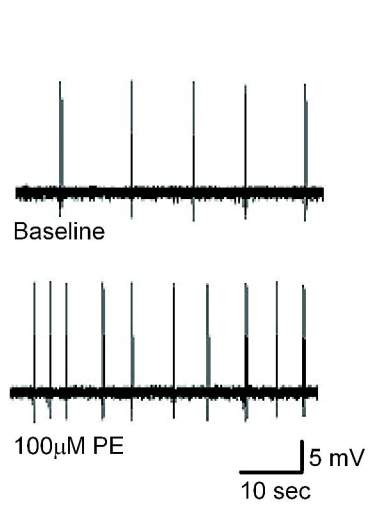

B.
Baseline
100μM PE
5 mV
10 sec

FIGURE 2.

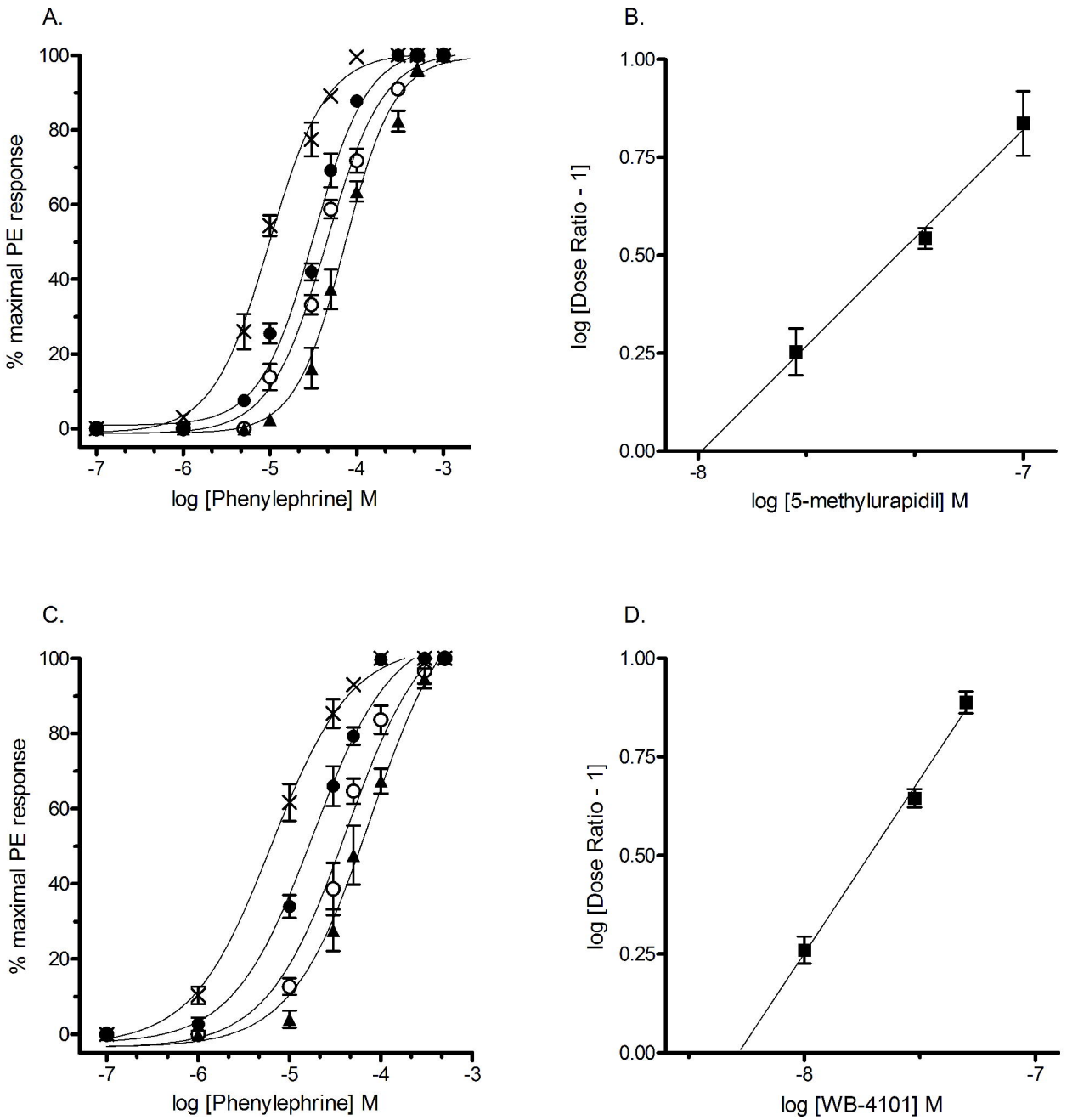

A.
% maximal PE response
log [Phenylephrine] M
B.
log [Dose Ratio - 1]
log [5-methylurapidil] M
C.
% maximal PE response
log [Phenylephrine] M
D.
log [Dose Ratio - 1]
log [WB-4101] M

FIGURE 3.

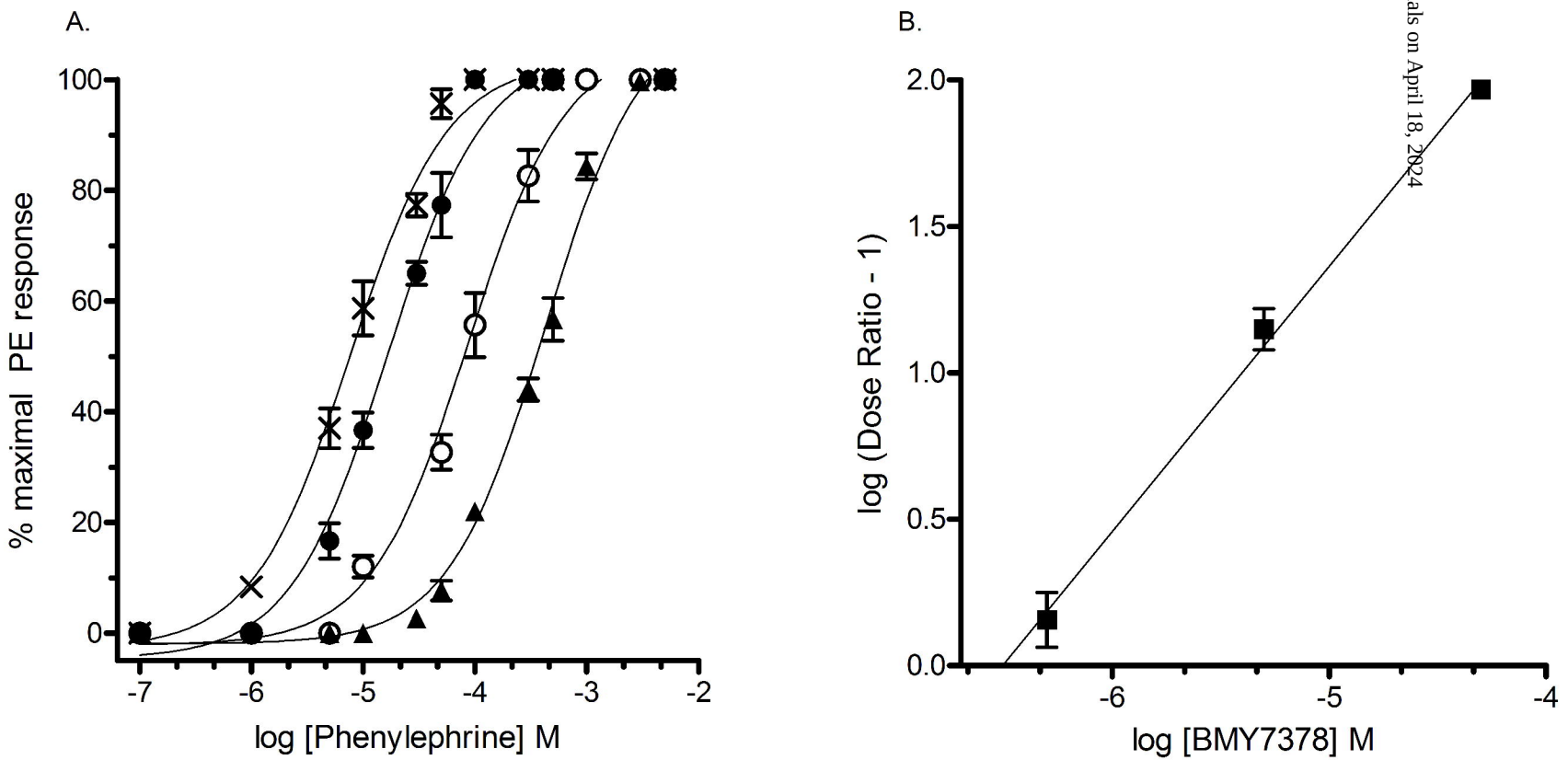


FIGURE 4.