Keith Parker:

PHARMACOLOGY OF BACOPA AT 5HT1A AND 5HT2A RECEPTORS

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Abstract

Bacopa monniera (Brahmi) is a perennial plant from India that has been long-used in the Ayurvedic system of medicine. It is frequently used as an adjunct to improve cognitive function. Serotonin (5-hydroxytryptamine; 5HT) is an autacoid and neurotransmitter associated with many physiological functions, including memory. 5HT's actions are mediated by a family of receptors (R), most of which are G Protein-Coupled (GPC), seven transmembrane (7TM) R. We have studied the in vitro pharmacology of Bacopa in two of the 5HTR's, the cloned 5HT1aR and 5HT2aR. Bacopa extract displaces the highly specific 5HT1a agonist [^{3}H]8-OH-DPAT from membrane preparations of the human (H) 5HT1aR. It also displaces the antagonist [^{3}H] ketanserin from membranes containing the rat 5HT2aR but at two-fold lower potency than in the displacement experiments at H5HT1aR. Signal transduction has been studied at H5HT1aR. In this negatively coupled system, Bacopa decreases cAMP levels, previously elevated with forskolin, by over 50% compared to control. This property suggests that one or more compounds in Bacopa act as agonists at H5HT1aR. Bacopa extract (BaCognize®) was solubilized in ethanolic water, and small percentages of ethanol are carried through all procedures. Control experiments showed that ethanol's actions were indistinguishable from control. In view of important pharmacologic effects that Bacopa has for cognitive health, it is vital to identify the specific compounds responsible for the in vitro pharmacology observed in this project.

Introduction

While pharmacological attempts to improve memory and other thought functions often understandably focus on the cholinergic and glutamatergic systems, there is rationale to support exploration of serotonergic approaches (1). Ultimately successful cognitive enhancement may require targeting multiple transmitter systems, and the natural product, Bacopa (5,6), may be poised nicely to do just that. Studies have shown that Bacopa is active with respect to other neurotransmitters (1,7), and with the evidence presented in this report, the serotonergic system can now be added to the list. While there are about 15 5HTR known currently, our laboratory has focused on two of these receptors, 5HT1aR and 5HT2aR (3,4). These two receptors have particularly prominent involvements in central nervous system disorders such as depression, anxiety, headache, and hallucination (2). Thus, the results presented from this project suggest possible therapeutic implications for bacopa's actions in memory enhancement utilizing 5HT1aR mechanisms and very likely 5HT2aR mechanisms. The future challenge is not only to identify and further characterize specific compounds in bacopa underlying these actions, but to expand knowledge of specific aspects of signal transduction modulated by bacopa's actions on these receptors. Additionally, other serotonin receptors beyond the scope of this study deserve exploration as this natural product appears to be diverse in its pharmacologic applications.

Methods

Cell Culture: Chinese Hamster Ovary (CHO) cells expressing H5HT1aR (4) were cultured in Ham's F-12 medium (GIBCO) fortified with 10% fetal bovine serum (Hyclone) and 200 ug/ml geneticin (Calbiochem). They were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were sub-cultured (trypsin, 0.25% in phosphate-buffered saline) or assayed upon confluency (5-8 days). Cloned H5HT1aR was a gift of Dr. John Raymond (Medical U. of S. Carolina). NIH 3T3 cells expressing the rat 5HT2aR (3) were cultured under similar conditions in DMEM (GIBCO) fortified with 10% calf serum (Hyclone) and 200 ug/ml geneticin. These cells were the gift of Dr. David Julius (UCSF). Both cell lines have been tested for mycoplasma with a PCR kit (ATCC), and are free of contamination.

Receptor Preparation: Cells were harvested in trypsin and centrifuged at low speed in ice-cold medium. The pellet was re-suspended in Earle's Balanced Salt Solution (GIBCO) followed by a second refrigerated sedimentation. Rinsed cells were then re-suspended in 10 ml of binding buffer (50 mM Tris, 4 mM CaCl2, 10 µM pargyline, pH 7.4), homogenized with Teflon-glass, and centrifuged at 450,000 g-min. at 4°C. To produce a crude membrane preparation, the pellet was finally re-suspended in 30 ml of ice-cold binding buffer, and re-homogenized, first with Teflon-glass and then with a Polytron (setting 4) for 5 sec. The receptor preparation was stored on ice and assayed within the next 1.5 hr.

Assay of Ligand-Receptor Binding: Binding of the agonist [^{3}H]8-OH-DPAT (NEN) to H5HT1aR followed well-characterized in vitro protocols (4). 1 ml reaction mixtures, in triplicate, were incubated for 30 min. in a 30°C shaker bath. Composition of the reaction mixture was: 700 µl of receptor homogenate; 100 µl of either binding buffer (total binding) or 10 µM 5HT (non-specific binding); 100 µl of triturated agent (0.5 nM); and 100 µl of diluted bacopa extract or binding buffer (control). Reactions were stopped in 4 ml of ice-cold Tris buffer and subsequent vacuum filtration on glass fiber filters (Whatman GF/B). Filters were rinsed twice in 5 ml of ice-cold Tris buffer, dried, and counted in 5 ml Ecoscint (National Diagnostics) in a Beckman LS 6500 counter. Homogenates were assayed for protein to maintain a nominal value of 50 µg per filter. Assays of the 5HT2aR (3) were similar except that mianserin (10 µM) was used for non-specific binding, and the triturated agent [^{3}H]ketanserin (NEN) was present at 0.2 nM; filtration utilized S & S #32 glass fiber filters.

cAMP Assay: CHO cells were cultured to confluency in 24-well plates. Cells were rinsed twice in warm, serum-free F-12 medium and incubated for 20 min. at 37°C in 0.5 ml of serum-free F-12 containing: 100 µM isobutylmethylxanthine (Sigma), 30 µM forskolin (ICN), and one or more of the following: 5HT (1 µM); bacopa extract (1/200); the 5HT1aR antagonist NAN-190 (50 nM). Reactions were stopped by aspiration and addition of 0.5 ml 100 mM HCl. After 10 min, well contents were harvested and centrifuged at 4,000 rpm. Supernatants were diluted in 100 mM HCl, and cAMP was quantified directly in a microplate format by colorimetric enzyme immunoassay with a kit from Assay Designs (Ann Arbor).

Dilution of Bacopa Extract: Powdered bacopa extract (BaCognize) was kindly provided by GeniHerbs. The powder (0.5g) was suspended in 10 mls of 70% ethanol (EtOH). The suspension was extracted overnight at room temperature with occasional stirring. Aliquots of fresh suspension were then diluted the day of assay, first in distilled water and then in buffer for final inclusion in the assays. Ethanol at the highest concentrations was not a factor in the assays (Fig. 2). BaCognize is estimated to contain 45% bacosides. Assuming complete extraction, the following concentrations of bacosides are estimated for the various dilutions: ethanol extract (0.0225 g/ml); and for the various dilutions of the extract all in µg/ml: 1/100 (250); 1/200 (125); 1/1000 (25); 1/5000 (5); 1/10,000 (2.5).

Figure 1. *Bacopa monnieri* (Brahmi)

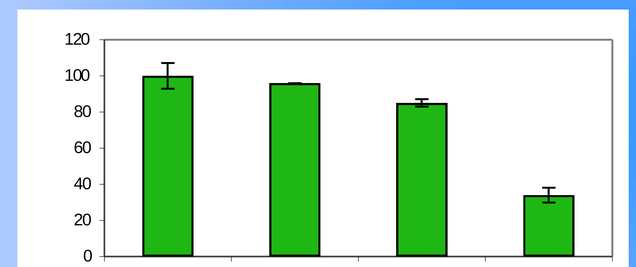

Figure 2. A comparison of the BaCognize® extract solublized in water or ethanol showing binding at the 5HT2a receptor. A test to determine the effects of ethanol on the binding profile of the bacopa extract did not differ significantly from the control. Extract dilutions: 1/100; n's 4-6.

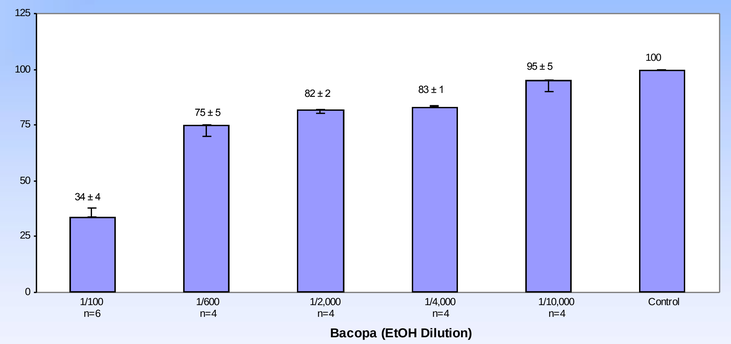

Figure 3. Binding of bacopa at 5HT2a receptor. Five ethanolic dilutions of the extract were tested for binding. n's 4-6.

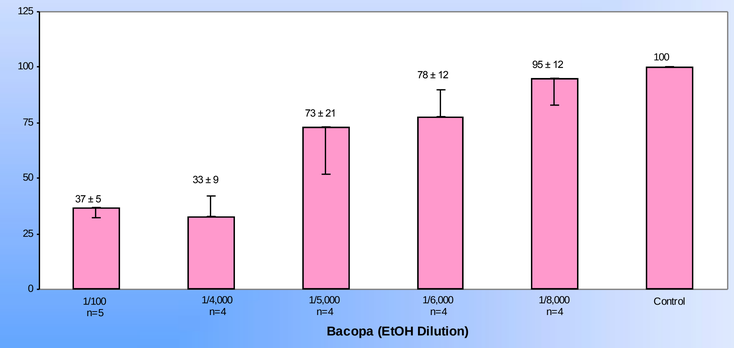

Figure 4. Binding of bacopa at human 5HT1a receptor. Five ethanolic dilutions of the extract were tested for binding; n's 4-5.

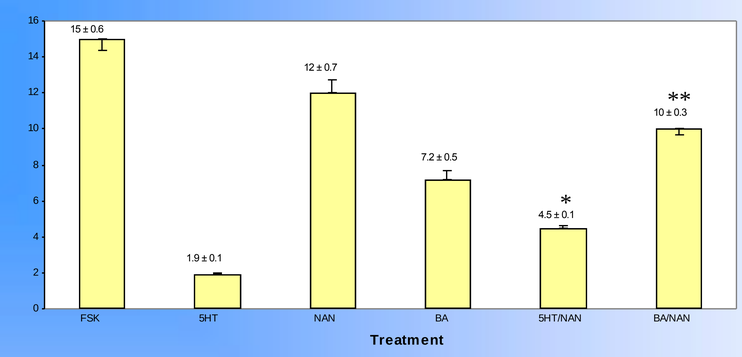

Figure 5. cAMP Assay. Bacopa (BA; 1/200) was tested for its ability to affect cAMP levels in vitro. Bacopa significantly decreased intracellular cAMP levels after pre-treatment with forskolin (FSK). NAN is a 5HT1aR antagonist. * p<0.01 5HT vs. 5HT/NAN ** p<0.01 BA vs. BA/NAN; n's 3-9.

Conclusions

1. Bacopa extract displaces [^{3}H]Ketanserin from rat 5HT2a receptors.
2. Ethanol solubilized extract contains more of bacopa's active constituents in 5HT2a receptor binding than the water solubilized extract.
3. Ethanol solubilized extract of bacopa displaces [^{3}H]8-OH-DPAT from human 5HT1a receptors. Bacopa is more potent in the 5HT1a receptor displacements compared to the 5HT2a displacements.
4. Ethanol solubilized extract of bacopa decreases cAMP production in cells expressing 5HT1a receptors; suggesting an agonistic effect.
5. The serotonergic system may play a role in bacopa's memory enhancing activity.

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