available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

# 8-OH-DPAT attenuates isoproterenol- but not forskolin-stimulated accumulation of cAMP in mediobasal hypothalamus

*Devi Majumdar, Angela Peterson-Ford, Lynda Uphouse**

*Department of Biology, Texas Woman's University, Denton, TX 76204, USA*

ARTICLE INFO

*Article history:*
Accepted 27 December 2005
Available online 9 February 2006

*Keywords:*
Tissue slice
Hypothalamus
Female rat
G protein
5-$HT_{1A}$ receptor

ABSTRACT

Ovariectomized female rats were used to test the possibility that the 5-$HT_{1A}$ receptor agonist, 8-hydroxy-2-(di-*N*-propylamino) tetralin (8-OH-DPAT), inhibits cyclic AMP (cAMP) accumulation in the mediobasal hypothalamus. Tissue slices were incubated with forskolin or with the β-adrenergic receptor agonist, isoproterenol, to stimulate accumulation of cAMP. Both compounds increased accumulation of cAMP. The 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT, reduced cAMP accumulation after stimulation by isoproterenol, but not after forskolin stimulation. These findings are discussed in terms of putative differences in the mechanisms whereby 5-$HT_{1A}$ receptors are able to inhibit stimulation of adenylate cyclase. The potential significance of these findings to 5-$HT_{1A}$ receptor-mediated inhibition of female rat lordosis behavior is also discussed.



## 1. Introduction

In previous experiments, we have shown that agonist activation of serotonin 1A (5-$HT_{1A}$) receptors in the mediobasal hypothalamus (MBH) inhibits lordosis behavior of sexually receptive female rats (Uphouse, 2000). The lordosis reflex is a supraspinal reflex that a sexually receptive female makes in response to sensory stimuli from the male (Pfaff and Modianos, 1985), and the ventromedial nucleus of the hypothalamus (VMN, located within the MBH) plays a critical role in this behavior (Pfaff and Modianos, 1985). An increase in neuronal firing within the VMN occurs when lordosis behavior is increased; and decreased firing within the VMN is associated with a reduction in lordosis behavior (Harlan et al., 1983; Kow et al., 1992). Consistent with findings that local application of estrogen to the VMN facilitates lordosis behavior (Rubin and Barfield, 1983), estrogen increases the overall excitability of VMN neurons (Kow and Pfaff, 1985). In contrast, agonist activation of 5-$HT_{1A}$ receptors within the VMN decreases neuronal firing (Kow et al., 1992).

5-$HT_{1A}$ receptors are G-protein-coupled receptors that can couple to a variety of effectors (Barnes and Sharp, 1999; Raymond et al., 1999). The best recognized consequence following 5-$HT_{1A}$ receptor activation is inhibition of adenylate cyclase (AC) and a reduced accumulation of cyclic AMP (cAMP) (DeVivo and Maayani, 1986; DeVivo and Maayani, 1990; Kushwaha and Albert, 2005; Raymond et al., 1999; Yocca et al., 1992). Agents that increase cAMP within the VMN can increase lordosis behavior, while agents that decrease cAMP within the VMN inhibit the behavior (Kow et al., 1994). Therefore, 5-$HT_{1A}$ receptors may reduce lordosis behavior by reducing cAMP. Consistent with this suggestion is the finding that forskolin, 8-bromo-cAMP, or dibutryl cAMP (all compounds which either increase or mimic cAMP accumulation)

* *Corresponding author*. Fax: +1 940 898 2382.
E-mail address: LUphouse@mail.twu.edu (L. Uphouse).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.12.111

attenuate the lordosis-inhibiting effects of a 5-$HT_{1A}$ receptor agonist, (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), on lordosis behavior (Uphouse et al., 2000). However, 5-$HT_{1A}$ receptor agonists do not inhibit cAMP accumulation in all brain areas (Billecocq et al., 1994; Clarke et al., 1996; Johnson et al., 1997).

To our knowledge, there is only one report in which 5-$HT_{1A}$ receptor-mediated effects on cAMP accumulation in hypothalamic tissue have been examined, and that report was in male rats and in membranes preparations (Billecocq et al., 1994). Since preparation of such membranes disrupts neuronal circuits, it is unclear whether or not these findings can be generalized to 8-OH-DPAT's effects on lordosis behavior. Infusions (whether of 5-$HT_{1A}$ receptor agonists or activators of cAMP) into the MBH are likely to influence a large number of neurons. Furthermore, since G protein specificity is more evident in intact cells than in vitro (Albert and Robillard, 2002; Gudermann et al., 1996), it is not clear whether or not 8-OH-DPAT would produce a decrease in cAMP accumulation within an intact MBH. Therefore, in the following experiments, we have tested the possibility that 8-OH-DPAT, by activating 5-$HT_{1A}$ receptors, reduces accumulation of cAMP in brain slices from the MBH.

## 2. Results

Over the dose range examined, forskolin dose dependently increased cAMP accumulation in MBH slices (Fig. 1) (ANOVA for regression, $F_{1,41}$ = 209.5, $P \leq 0.0002$, $r$ = 0.91). A concentration of 100 μM forskolin was chosen to examine the effects of the 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT. Forskolin-stimulated cAMP accumulation was not affected by the 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT (ANOVA, $F_{3,18}$ = 1.78, $P > 0.05$) (Fig. 2). The effects of 8-OH-DPAT were then examined in slices stimulated with either 10 μM or 100 μM forskolin (Fig. 3). Regardless of the concentration of forskolin, there was not a significant effect of 8-OH-DPAT on forskolin-stimulated cAMP accumulation ($P > 0.05$).

Since forskolin can increase neuronal activity (Kaneko and Takahashi, 2004), it was possible that forskolin increased release of neurotransmitters that were positively coupled to AC and that 8-OH-DPAT was unable to block such transsynaptic accumulation of cAMP. To investigate this possibility, we incubated MBH slices in the presence or absence of 10 mM

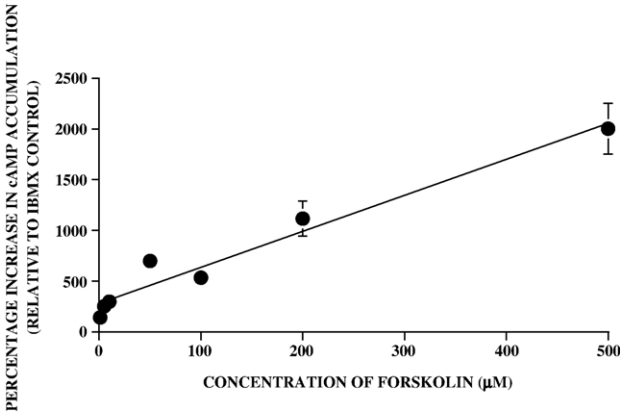


**Fig. 1 – Forskolin dose dependently increases cAMP accumulation in MBH. Data are the mean ± SE percentage increase in cAMP accumulation following incubation with varying concentrations of forskolin. Picomoles cAMP/μg protein were computed (within assay days) as a percentage of the cAMP accumulation in the IBMX control (no forskolin). *N*s for 0, 1, 5, 10, 50, 100, 200, and 500 μM forskolin, respectively, are 11, 6, 6, 11, 3, 6, 5, and 3. The computer-generated line represents the line of best fit for the data (Cricket Graph III, v 1.53, Computer Associates International, Inc.).**

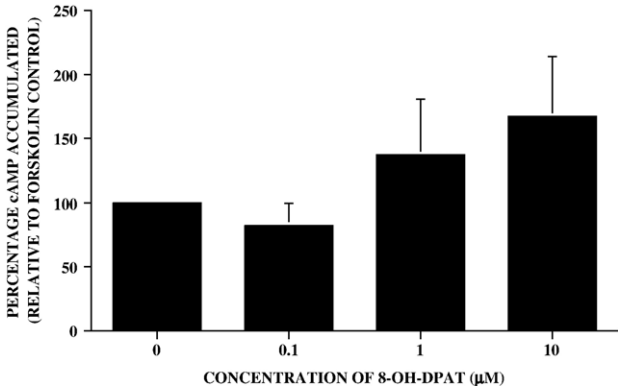


**Fig. 2 – Effect of 8-OH-DPAT on forskolin-stimulated cAMP in MBH. MBH slices were incubated as described in the Experimental procedures with 100 μM forskolin plus varying concentrations of 8-OH-DPAT. Data are the mean ± SE percentage of cAMP relative to the 100 μM forskolin control (0 μM 8-OH-DPAT), within assays. The *N*s for 8-OH-DPAT concentrations of 0, 0.1, 1.0, and 10 μM, respectively, are 7, 4, 4, and 7. The mean ± SE pmol cAMP/μg protein with forskolin, alone, was 0.027 ± 0.008.**

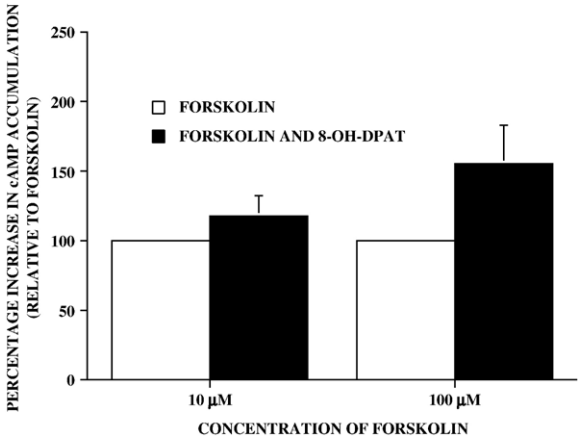


**Fig. 3 – Effects of 8-OH-DPAT on cAMP accumulation stimulated by 10 or 100 μM forskolin. MBH slices were incubated as described in the Experimental procedures with 10 μM or 100 μM forskolin plus 10 μM 8-OH-DPAT. Data are the mean ± SE percentage of cAMP relative to the appropriate concentration of forskolin control. Data are the results from 4 different animals.**