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Factors Elevating cAMP Attenuate the Effects of 8-OH-DPAT on Lordosis Behavior

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UPHOUSE, L., S. MASWOOD AND A. JACKSON. *Factors elevating cAMP attenuate the effects of 8-OH-DPAT on lordosis behavior.* PHARMACOL BIOCHEM BEHAV **66**(2) 383–388, 2000.—The effects of a soluble derivative of forskolin and of two membrane-permeable analogs of cAMP, dibutyryl cAMP, and 8-bromo-cAMP, on the ability of a serotonin (5-HT)$_{1A}$ receptor agonist to inhibit lordosis behavior were examined. Sexually receptive, proestrous rats received a bilateral infusion into the ventromedial nucleus of the hypothalamus (VMN) with 68 ng of the forskolin derivative 1, 1.5, 2, or 2.5 h prior to infusion with 200 ng of the 5-HT$_{1A}$ receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Proestrous rats and ovariectomized rats, hormonally primed with 25 μg estradiol benzoate and 500 μg progesterone, were coinfused with 200 ng 8-OH-DPAT and either 50 μg dibutyryl cAMP or 5 μg 8-bromo-cAMP. In proestrous rats, prior infusion with the forskolin derivative reduced the effects of the 5-HT$_{1A}$ receptor agonist on lordosis behavior. The best protection occurred at 2 h; by 2.5 h after the preinfusion, any protective effect had disappeared. Coinfusion with either dibutyryl-cAMP or 8-bromo-cAMP reduced the effects of 8-OH-DPAT in proestrous rats. In hormone-primed, ovariectomized rats, dibutyryl cAMP offered significant protection against the effects of 8-OH-DPAT, but there was no protection with 8-bromo-cAMP. These findings are consistent with the speculation that 8-OH-DPAT's inhibition of lordosis behavior results, in part, from an inhibition of adenylyl cyclase, resulting from agonist activation of 5-HT$_{1A}$ receptors in the VMN. The findings are also consistent with our earlier observations for differences between proestrous rats and hormone-primed, ovariectomized rats in their response to 5-HT receptor-active compounds. © 2000 Elsevier Science Inc.

Dibutyryl cAMP Forskolin 8-Bromo-cAMP Female rats 5-HT$_{1A}$ receptor Sexual behavior

SINCE Meyerson (23) hypothesized that serotonin (5-HT) inhibited lordosis behavior, there has been an accumulation of evidence that (a) treatments which reduce 5-HT activity increase lordosis behavior (21,22); (b) treatments that increase 5-HT activity reduce lordosis behavior (20,22); and (c) the mediobasal hypothalamus (MBH) [especially the ventromedial nucleus of the hypothalamus (VMN)] is a site where such 5-HT-mediated inhibition occurs (7,20,32,33). It is now clear that 5-HT$_{1A}$ receptors are involved in the inhibition of lordosis behavior (1,2,8,32,33), that 5-HT$_{1A}$ receptors are present in high to moderate density throughout the VMN (25), and that intracranial infusion with 5-HT$_{1A}$ receptor agonists inhibits lordosis behavior (9,32,33). The mechanisms responsible for this effect of 5-HT$_{1A}$ receptor agonists, however, are not known.

5-HT produces biphasic effects on neuronal firing in many brain areas (3,17), and in tissue slices containing the VMN, the 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), mimicked 5-HT's inhibitory effect on neuronal firing (17). Thus, the lordosis-inhibiting effects of the 5-HT$_{1A}$ receptor agonists is consistent with evidence that an increase in firing of VMN neurons occurs when lordosis behavior is increased, while reduced firing of VMN neurons occurs under conditions where the behavior is decreased (10,15,16,27). Kow and Pfaff (15) reported that estrogen enhanced the overall excitability of VMN neurons, and speculated that this excitability contributed to the hormone's facilitation of lordosis behavior. However, estrogen may also stimulate cAMP accumulation in the hypothalamus (9,16), and has been shown to stimulate adenylyl cyclase and cAMP-regulated gene transcription in peripheral estrogen-responsive tissues (4). Agents that increase adenylyl cyclase in the VMN facilitate lordosis behavior, while agents that inhibit adenylyl cyclase in the VMN reduce lordosis behavior (16). Because 5-HT$_{1A}$ receptors are negatively coupled to adenylyl cyclase (6,11,26,30), an inhibition of the accumulation of cAMP may contribute to 5-HT's inhibition of lordosis behavior. If so, elevation of cAMP should protect against the lordosis-inhibiting effects of 5-HT$_{1A}$ receptor agonists.

In the following experiments, we have tested the hypothesis that compounds that elevate cAMP protect against the ef-

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fects of 8-OH-DPAT on lordosis behavior. A water-soluble derivative of forskolin {7β-deacetyl-7β-[γ-(morpholino)butyryl]-hydrochloride} was used to elevate cAMP (18); dibutyryl cAMP and 8-bromo-cAMP, membrane-permeant analogs of cAMP (24), were used to simulate effects of an elevation in cAMP.

METHOD

Materials

(±)8-Hydroxy-2-(di-n-propylamino)tetralin, hydrogen bromide (8-OH-DPAT), a soluble derivative of forskolin {7β-deacetyl-7β-[γ-(morpholino)butyryl]-hydrochloride} and 8-bromo cAMP (8-bromoadenosine-3′, 5′-cyclophosphate sodium) were purchased from Research Biochemicals International (Natick, MA). Dibutyryl cAMP (N6,2′-o-dibutyryladenosine-3′: 5′-cyclic monophosphate), estradiol benzoate, and progesterone were obtained from Sigma Chemical Co. (St. Louis, MO). Intracranial cannulae were obtained from Plastic Products, Inc. (Roanoke, VA), and dental acrylic was obtained from Reliance Dental Mfg. Co. (Worth, IL). Suture material was obtained from Butler Co. (Arlington, TX), and methoxyflurane (Metofane®) was purchased from Pitman Moore (Mundelein, IL). All other materials were purchased from Fisher Scientific (Houston, TX).

Animals and Housing Conditions

Adult, female rats (CDF-344) were purchased from Sasco Laboratories, or were bred in the TWU animal facility from stock obtained from Sasco Laboratories (Wilmington, MA). Purchased rats were housed for at least 2 weeks prior to the studies. Rats, bred in the TWU facility, were weaned at approximately 30 days of age. Rats were housed in groups of three with like-sex littermates in polycarbonate cages with food and water available ad lib. The housing rooms were maintained at 22°C with 55% humidity under a reversed light–dark cycle with lights off from 1200 to 2400 h.

Surgical Procedures and Treatment of Animals

At approximately 90 days of age, rats were anesthetized with Metofane® and implanted bilaterally with 22-gauge stainless steel guide cannulae advanced stereotactically toward the VMN [atlas coordinates from Konig and Klippel (14) AP 4.38; DV 7.8; ML 0.4] as previously described (32,33). Guide cannulae were secured with dental acrylic and anchored to the skull with three stainless steel screws. Stainless steel dummy cannulae, terminating approximately 0.5 mm below the guide cannulae, were placed in the guide cannulae at the time of implant surgery to prevent clogging.

When intact, proestrous rats were used, vaginal smearing began approximately 2 weeks after implant surgery. Rats displaying a proestrous smear and lordosis/mount ratios = 0.8 on the day of testing were used in the experiment. Vaginal smearing was performed between 1000 and 1100 h (1–2 h before the onset of the dark portion of the light/dark cycle). At this time, vaginal smears from rats that will be sexually receptive that afternoon are characterized by a high proportion of nucleated cells (often clumped in appearance).

When ovariectomized rats were used, rats were bilaterally ovariectomized 2 weeks after the VMN implant procedure. Surgery took place under Metofane® anesthesia as previously described (12,31). Hormonal priming began 2 weeks after ovariectomy.

After all surgeries, rats were monitored until recovery to assure the animal's welfare. All experimental procedures were approved by the TWU Animal Care and Use Committee.

Behavioral Testing Procedures

Testing for sexual receptivity took place during the dark portion of the light/dark cycle as previously described (33). For sexual behavior testing prior to drug treatments, each female was placed with a sexually experienced male rat in his home cage. The male was allowed to mount the female 10 times, and sexual receptivity was determined. After drug infusions, data were grouped into 5-min intervals. Attempts were made to prevent males from ejaculating during the test period. If ejaculation occurred, the male was removed and the female was left undisturbed for the remainder of the 5-min interval. The female was then introduced to a new male for any remaining intervals. Sexual receptivity data for the pretest and each of the test intervals were quantified as the lordosis/mount (L/M) ratio. Lordosis quality, rejection behavior, and incidences of hopping and darting were also recorded.

Drug Infusion Procedures

Dummy cannulae were replaced with 28-gauge stainless steel internal cannulae attached by tubing (i.d. = 0.58 mm; o.d. = 0.96 mm) to a CMA/100 (Bioanalytical Systems, Lafayette IN) microinjector. Infusions were administered at a flow rate of 0.24 to 0.26 μl/min to a final infusion volume of 0.5 μl/site. After testing was complete, cannulae locations were determined as previously described (32,33).

SPECIFIC EXPERIMENTS

Experiments with Intact, Proestrous Rats

Effects of the soluble derivative of forskolin. A total of 45 proestrous rats were used in the experiment. Thirty-five rats were infused with 68 ng of the water soluble derivative of forskolin 1 h (n = 8), 1.5 h (n = 9), 2 h (n = 8), or 2.5 h (n = 10) prior to infusion with 200 ng 8-OH-DPAT. Four rats (one from the 1-h group, two from the 1.5-h group, and one from the 8-OH-DPAT–only group) would not allow the male to mount after infusion with 8-OH-DPAT, and were excluded from statistical analyses; one rat (1.5-h group) had an L/M ratio = 0 before infusion with 8-OH-DPAT, and was also excluded. The forskolin derivative was dissolved in deionized water and was delivered in a volume per bilateral cannula of 0.5 μl. Five rats were preinfused with 0.5 μl deionized water and were infused with 200 ng 8-OH-DPAT 1 to 2.5 h later. Five rats received no preinfusion, but were infused with 200 ng 8-OH-DPAT. There was no differences between rats preinfused with water and those that received no infusion prior to 8-OH-DPAT, so the data were combined for analysis.

Rats were gently hand restrained during the preinfusion with the forskolin derivative or with water. Infusion with 8-OH-DPAT occurred within a CMA/120 containment system (Bioanalytical Systems) as previously detailed (33). The female was allowed to adapt to the chamber for 5–10 min before a sexually active male was placed in the chamber. Sexual behavior of the female was recorded prior to the infusion with 8-OH-DPAT, during the infusion, and for 30 consecutive min after the infusion.

Effects of dibutyryl cAMP and 8-bromo-cAMP. In the second experiment, proestrous rats were infused with 200 ng 8-OH-DPAT (n = 12), were coinfused with 8-OH-DPAT and

50 μg of dibutyryl cAMP (n = 5), or were coinfused with 8-OH-DPAT and 5 μg of 8-bromo-cAMP (n = 10). Coinfusions with 8-OH-DPAT and 8-bromo-cAMP were conducted while the female was in the CMA/120 containment system. Coinfusions with 8-OH-DPAT and dibutyryl cAMP were performed while the female was gently hand restrained. Infusions with 8-OH-DPAT alone were performed in both manners to match the coinfusion procedures. Because there were no differences between the two 8-OH-DPAT infusion procedures, data were combined for analysis.

After a pretest with the sexually active male, the female was infused with the test compound(s) as described above. Cannulae were left in place for 2 min before removal. Testing for sexual behavior, as described above, began 10 min after the infusion.

Experiments With Hormone-Primed, Ovariectomized Rats

Two weeks after ovariectomy, rats were injected subcutaneously (SC) with 25 μg estradiol benzoate (in sesame seed oil) in a volume of 0.1 ml/rat. Approximately 48 h later, the rats received 500 μg progesterone (in propylene glycol, SC, 0.1 ml/rat). Testing for sexual receptivity took place 4–6 h after the progesterone injection and occurred during the dark portion of the light–dark cycle. After a pretest for sexual receptivity, rats were infused with 200 ng 8-OH-DPAT (n = 18), were coinfused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP (n = 10), or were coinfused with 200 ng 8-OH-DPAT and 8-bromo-cAMP (n = 8) as described for proestrous rats. Postinfusion testing began 10 min after the infusion.

Because we have previously reported that ovariectomized rats primed for 2 consecutive weeks with estradiol benzoate show little, if any, decline in lordosis behavior after infusion with 200 ng 8-OH-DPAT, an additional group was included for comparison to the effects of dibutyryl cAMP and 8-bromo-cAMP in rats that received only one injection with estradiol benzoate. These rats (EEP, n = 14) were injected with 25 μg estradiol benzoate 2 weeks after ovariectomy. One week later, the estradiol benzoate injection was repeated and 500 μg progesterone were injected 48 h later. The effects of bilateral VMN infusion of 200 ng 8-OH-DPAT were examined 4–6 h after the progesterone injection.

Statistical Procedures

Lordosis quality and lordosis frequency [quantified as the number of lordosis responses divided by the number of mounts by the male (L/M ratio)] were organized into pretest period, infusion period (if appropriate), and consecutive 5-min intervals during the behavioral testing. Data were evaluated by repeated measures ANOVA with the statistical software package Super ANOVA 1.1® from Abacus Concepts Inc. (Calabasas, CA). Post hoc comparisons were performed with either Dunnett's test or Tukey's test. In all cases, the statistical reference was Zar (34), and an alpha level of 0.05 was required for rejection of the null hypothesis.

RESULTS

Intact, Proestrous Rats

The effect of preinfusion with the soluble derivative of forskolin on the later response to 8-OH-DPAT is shown in Fig. 1. Bilateral VMN infusion with 200 ng 8-OH-DPAT inhibited lordosis behavior and there was a significant protective effect of prior infusion with the forskolin derivative. Treatment, $F(4, 32) = 2.68$, $p \leq 0.05$, and time after infusion with 8-OH-DPAT, $F(7, 224) \leq 9.21$, $p \leq 0.0001$, were significant. Although the best protection occurred 2 h after preinfusion with the forskolin derivative, some protection was evident earlier. By 2.5 h after infusion with the forskolin derivative, any protective effect had disappeared. This time-dependent effect of the preinfusion is shown in Fig. 2.

Although there were also significant treatment effects on lordosis quality, $F(4, 19) = 3.60$, $p \leq 0.024$, neither the time after 8-OH-DPAT nor the interaction term was significant (data not shown). Mean ± SE lordosis quality over the entire testing period for rats infused with 8-OH-DPAT or preinfused with the forskolin derivative 1, 1.5, 2, or 2.5 h earlier, respectively, were 2.64 ± 0.065, 2.98 ± 0.056, 2.77 ± 0.068, 3.04 ± 0.027, and 2.92 ± 0.055.

The effects of dibutyryl cAMP and 8-bromo-cAMP on the response to 8-OH-DPAT in proestrous rats are shown in Fig. 3. There were significant effects of treatment, $F(2, 24) \leq 3.50$, $p \leq 0.05$, and time after infusion, $F(5, 120) = 8.20$, $p \leq 0.0001$; the interaction term narrowly escaped statistical significance, $F(10, 120) = 1.85$, $p \leq 0.059$. Similar to the protective effects

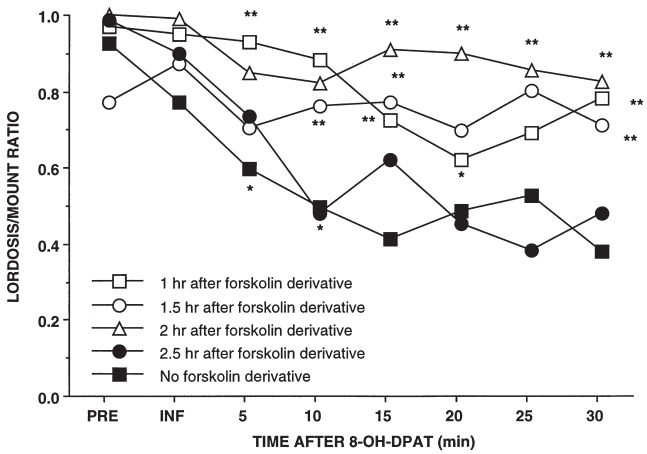

FIG. 1. Preinfusion with a soluble derivative of forskolin attenuates the effects of 8-OH-DPAT. Forty-five proestrous rats received bilateral VMN infusion with 200 ng 8-OH-DPAT. Thirty-five rats were preinfused with 68 ng of the water soluble derivative of forskolin {7β-deacetyl-7η-[γ-(morpholino) butyryl]-hydrochloride} 1, 1.5, 2, or 2.5 h prior to infusion with 200 ng 8-OH-DPAT. Four rats (one from the 1-h group, two from the 1.5-h group, and one from 8-OH-DPAT–only, group) would not allow the male to mount after infusion with 8-OH-DPAT, and were excluded from the data presentation; one rat (1.5-h group) had an L/M ratio = 0 before infusion with 8-OH-DPAT, and was also excluded. Shown in the figure are the mean L/M ratios for nine females infused with 8-OH-DPAT without prior infusion with the forskolin derivative. Also shown are the ratios for rats infused with the forskolin derivative 1 (n = 7), 1.5 (n = 6), 2.0 (n = 1 > n = 7), or 2.5 h (n = 8) prior to infusion with 8-OH-DPAT. Data are L/M ratios for the pretest interval, infusion period, and for six consecutive 5-min intervals after infusion with 8-OH-DPAT. The SE bars were omitted for ease of viewing, but the overall SE for 8-OH-DPAT only, and for the 1-, 1.5-, 2-, and 2.5-h preinfusion groups, respectively, was 0.043, 0.042, 0.041, 0.036, and 0.047. Single asterisks indicate the first 5-min interval that the L/M ratio for an individual group was significantly less than the pretest interval (all Dunnett's q224, 8 = 2.61, all $p \leq 0.05$). Double asterisks indicate intervals where the L/M ratios were significantly different from rats infused with 8-OH-DPAT without prior infusion with the forskolin derivative (all Tukey's q224,4 = 3.32, $p \leq 0.05$).

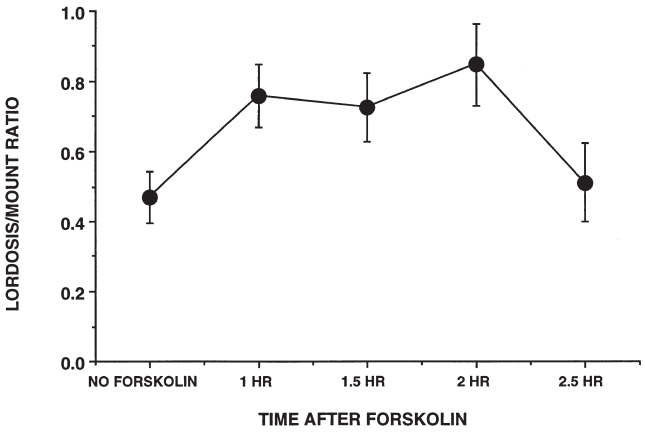

FIG. 2. Time-dependent effects of forskolin on the response to 8-OH-DPAT. From the data in Fig. 1, the grand mean for the 5–30-min intervals after infusion were computed for each rat. In Fig. 2, the mean ± SE of these grand means for the various treatment groups are shown as a function of time between infusion with forskolin and infusion with 8-OH-DPAT.

of forskolin, both dibutyryl cAMP and 8-bromo-cAMP attenuated the lordosis-inhibiting effects of 8-OH-DPAT.

Hormone-Primed, Ovariectomized Rats

In agreement with previous findings (12,31), 200 ng 8-OH-DPAT significantly reduced L/M ratios of ovariectomized rats primed once with 25 μg estradiol benzoate and 500 μg progesterone (EP), and treatment with 25 μg estradiol benzoate 1 week earlier (EEP) significantly attenuated the effects of the 5-HT_{1A} receptor agonist (Fig. 4). Dibutyryl cAMP, but not 8-bromo-cAMP, reduced the effects of 8-OH-DPAT in the EP-primed ovariectomized rats (Fig. 4). There were significant effects of treatment, $F(3, 46) = 21.48$, $p \leq 0.0001$, time after infusion, $F(5, 230) = 30.70$, $p \leq 0.0001$, and a significant time × treatment interaction, $F(15, 230) = 5.51$, $p \leq 0.0001$. However, infusion with dibutyryl cAMP was less effective in reducing the response to 8-OH-DPAT than was the prior treatment with estradiol benzoate.

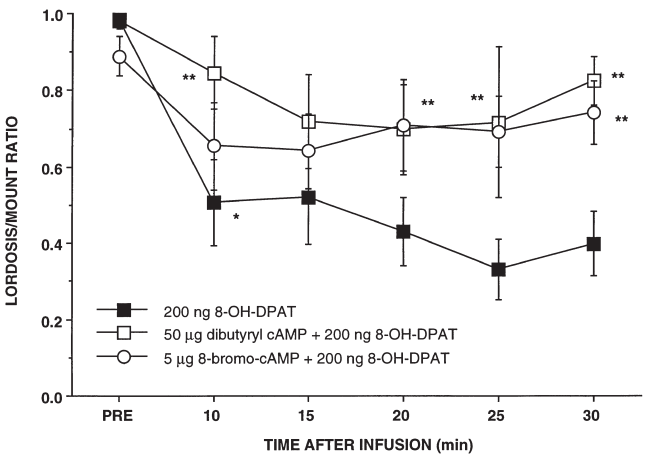

FIG. 3. Effects of dibutyryl cAMP and 8-bromo-cAMP on the response of proestrous rats to 8-OH-DPAT. Data are the mean ± SE L/M ratios for 12 rats infused into the VMN with 200 ng 8-OH-DPAT, 10 rats coinfused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP, and 5 rats coinfused with 200 ng 8-OH-DPAT and 5 μg 8-bromo-cAMP. Data are for the pretest interval and for consecutive 5-min test intervals conducted 10 through 30 min after the infusion. The single asterisk indicates the first 5-min intervals where L/M ratios of rats infused with 8-OH-DPAT differed significantly from their pretest interval (Dunnett's q120, 6 = 2.51, $p \leq 0.05$). Double asterisks indicate intervals where rats coinfused with dibutyryl cAMP or 8-bromo-cAMP and 8-OH-DPAT were significantly different from rats infused with 8-OH-DPAT, only (Tukey's q120,3 = 3.35, $p \leq 0.05$).

DISCUSSION

The inhibitory effect of 5-HT_{1A} receptor agonists on lordosis behavior has been thoroughly described, and the VMN is at least one brain area where such inhibition is mediated (2,21,31–33). The 5-HT_{1A} receptors belong to the superfamily of G-protein–coupled receptors (29), and activation of 5-HT_{1A} receptors is generally associated with inhibition of adenylyl cyclase or opening of a K+ channel (11,19). However, 5-HT_{1A} receptors have been suggested to couple to multiple second-messenger systems (6,13,19), so it remains unclear exactly what effect of 5-HT_{1A} receptors is responsible for the decline in female rat lordosis behavior.

If a 5-HT_{1A} receptor-mediated reduction of cAMP contributes to the effects of 5-HT_{1A} receptor agonists on lordosis behavior, then agents that increase cAMP should protect against the effects of the 5-HT_{1A} receptor agonists. In the present

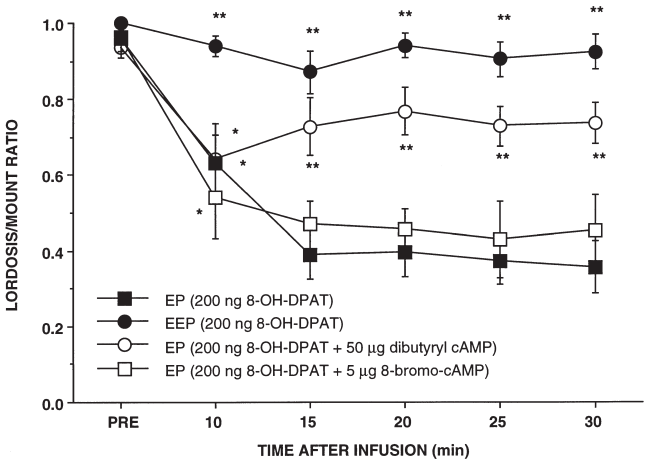

FIG. 4. Effects of dibutyryl cAMP and 8-bromo-cAMP on the response of hormone-primed, ovariectomized rats to 8-OH-DPAT. Ovariectomized rats with bilateral VMN implants were injected SC with 25 μg estradiol benzoate followed 48 h later with 500 μg progesterone. Eighteen rats were infused with 200 ng 8-OH-DPAT 4–6 h after the progesterone injection. Ten rats were coinfused with 200 ng 8-OH-DPAT and 50 μg dibutyryl cAMP; 8 rats were coinfused with 200 ng 8-OH-DPAT and 5 μg 8-bromo-cAMP. Data are the mean ± SE L/M ratios for rats during the pretest before VMN infusion and for consecutive 5-min intervals from 10 through 30 min after the infusion. Also shown in the figure are the mean ± SE L/M ratios for 14 ovariectomized rats that were primed with 25 μg estradiol benzoate 1 week before injection with 25 μg estradiol benzoate and 500 μg progesterone. These rats were infused with 200 ng 8-OH-DPAT. Single asterisks indicate the first 5 min interval where the L/M ratio for a group declined significantly relative to the pretest interval (Dunnett's q230,6 = 2.51, $p \leq 0.05$). Double asterisks indicate intervals where the L/M ratio was significantly different from 8-OH-DPAT, only (Tukey's q230,4 = 3.66, $p \leq 0.05$).

studies, all three compounds (forskolin derivative, dibutyryl cAMP, and 8-bromo-cAMP) reduced the effects of 8-OH-DPAT in proestrous rats. The protective effect of the forskolin derivative occurred gradually 1 to 2 h after its infusion. This delay is consistent with observations by other investigators that there is a delay of about 90 min between intracranial infusion with forskolin and an elevation of cAMP (28). The relatively short time period during which this protection was evident probably results from the rapid hydrolysis of the stimulated increase in cAMP and removal of the soluble forskolin derivative by diffusion from the injection site.

The relatively brief effect of the soluble forskolin derivative, coupled with the protective effects of dibutyryl cAMP and 8-bromo-cAMP following coinfusion with 8-OH-DPAT, leads to the speculation that an increase in cAMP must occur coincident with activation of 5-HT_{1A} receptors if the lordosis-inhibiting effects of these receptors is to be attenuated. Unfortunately, we were unable to test this hypothesis. When rats were infused with dibutyryl cAMP prior to infusion with 8-OH-DPAT, a large proportion of both proestrous females and hormone-primed, ovariectomized females developed severe seizures upon reinsertion of the infusion cannulae. Such seizures appeared to result from the insertion of the cannulae, and were not a consequence of drug infusion. Therefore, this study was discontinued.

In hormone-primed, ovariectomized rats, only dibutyryl cAMP and 8-bromo-cAMP were examined. In these rats, coinfusion with dibutyryl cAMP and 8-OH-DPAT produced less inhibition of lordosis behavior than was seen following infusion with 8-OH-DPAT alone. However, 8-bromo-cAMP did not protect against the effects of 8-OH-DPAT. This probably reflects the relatively small concentration (5 μg) of 8-bromo-cAMP that was infused in the experiment. Because 5 μg of 8-bromo-cAMP did protect against the effects of 8-OH-DPAT in proestrous rats, higher concentrations of 8-bromo-cAMP might have been effective in the hormone-primed, ovariectomized rats. The concentration of dibutyryl cAMP was based on a previous report by Beyer et al. (5) that 50 μg of dibutyryl cAMP infused into the preoptic area increased lordosis responding in estrogen-primed, ovariectomized rats. The chosen concentration of 8-bromo-cAMP was based on its solubility in water. We were unsuccessful in dissolving 8-bromo-cAMP in water at a concentration greater than the 5 μg/0.5 μl infusion volume. Higher concentrations could not be examined without either an increase in the infusion volume or the use of a nonaqueous vehicle.

We previously reported that bilateral VMN infusion of 200 ng 8-OH-DPAT inhibited lordosis behavior in a majority of proestrous rats while lower concentrations were less consistent in their effects (33). More recently, however, we found that lower concentrations of 8-OH-DPAT infused into the VMN could reduce lordosis behavior in a majority of ovariectomized rats primed with estradiol benzoate and progesterone (12). Similarly, Truitt et al. (29) have reported a reduction in L/M ratios in hormone-primed ovariectomized rats by intraperitoneal treatment with doses of 8-OH-DPAT that are ineffective in proestrous rats. Consequently, hormone-primed ovariectomized females may be more sensitive to the lordosis-inhibiting effects of 5-HT_{1A} receptor agonists. This might account for the differential effects of 8-bromo-cAMP in proestrous rats and in hormone-primed, ovariectomized rats. Clearly, further studies are needed to explore this possibility.

In summary, the present findings are consistent with evidence that 5-HT_{1A} receptors reduce accumulation of cAMP, and that compounds that reduce cAMP in the VMN reduce lordosis behavior. In the current studies, the lordosis-inhibiting effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, in proestrous rats were attenuated by three different compounds expected to simulate an elevation of cAMP. In hormone-primed, ovariectomized rats, a prior treatment with estradiol benzoate was, however, more effective than either dibutyryl cAMP or 8-bromo-cAMP in preventing the decline in lordosis behavior after VMN infusion with 200 ng 8-OH-DPAT. Consequently, hormone-primed, ovariectomized rats may be more responsive to the effects of the 5-HT_{1A} receptor agonists, may show a smaller response to the cAMP analogs, or may accumulate less cAMP than proestrous rats. Additional studies will be required to differentiate the effects of these compounds in proestrous and hormone-primed, ovariectomized rats. Nevertheless, the current studies provide evidence that 5-HT_{1A} receptors in the VMN reduce lordosis behavior, in part, by reducing accumulation of cAMP.

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