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Brain Research 711 (1996) 84–92

BRAIN RESEARCH

Research report

In vivo evidence for progesterone dependent decreases in serotonin release in the hypothalamus and midbrain central grey: relation to the induction of lordosis

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Received 6 September 1995; accepted 24 October 1995

Abstract

The effects of progesterone (P) on serotonin (5HT) overflow in the ventromedial hypothalamus (VMH), preoptic area (POA) and midbrain central grey (MCG) were studied using in vivo microdialysis. Ovariectomized rats, pretreated with 5 μg estradiol, were anesthetized with chloral hydrate and stereotaxically implanted with dialysis probes directed towards one of the respective brain sites. Extracellular 5HT levels stabilized 3 to 5 h following probe implantation. Under stable baseline conditions, perfusion of 1 μM tetrodotoxin through the dialysis probe resulted in a 60–65% reduction in 5HT overflow in the brain areas studied. In experiments testing the effect of P on 5HT overflow, rats were subcutaneously injected with 0.5 mg P or propylene glycol vehicle. Samples were analyzed for 5HT at 20 min intervals for 4 h after treatment. Perfusate levels of 5HT were not significantly changed in the VMH, POA or MCG in vehicle-treated rats. Similarly, P treatment failed to significantly alter 5HT overflow in the POA. In the VMH, perfusate levels of 5HT were significantly reduced 60 min after P treatment. Decreases in perfusate 5HT levels were detected 20 min after P in the MCG. The decreases in 5HT overflow measured in the VMH and MCG following P treatment persisted for the remainder of the sampling period with the exception of 1 time point in the VMH. The results provide in vivo evidence for P-influenced decreases in 5HT release in the VMH and MCG. The rapid decrease in extracellular 5HT in the MCG suggests that this effect may represent a non-genomic action of P. These results are discussed in relation to the role of 5HT in the regulation of lordosis behavior.

Keywords: Microdialysis; 5HT; Progesterone; Ventromedial hypothalamus; Preoptic area; Midbrain central grey; Lordosis

1. Introduction

The view that serotonin (5HT) exerts chronic inhibition on lordosis behavior has a long history of support from pharmacological studies [2,6,18,26,36,43]. The importance of 5HT in the regulation of female sexual behavior is further suggested by experiments demonstrating the presence of P-influenced changes in 5HT turnover in specific brain regions of the estrogen (E)-primed ovariectomized rat [14,16,33]. The results from these studies indicate that one of the actions of P is to decrease serotonergic activity in the ventromedial hypothalamus (VMH) [14,16,33] and midbrain central grey (MCG) [8,33], possibly releasing these sites from a tonic inhibitory serotonergic influence on lordosis behavior [18,20,33].

A series of recent pharmacological studies indicate that the role of 5HT in the regulation of female sexual behavior may be considerably more complex than simple tonic inhibition of behavior [1,10,13]. The hypothesis that 5HT serves a dual role in the control of lordosis emerged from studies demonstrating that sexual behavior in E- and P-treated rats can be inhibited by systemic administration of $5HT_2$ antagonists [24] and $5HT_1$ agonists [13,25], respectively. In addition, evidence suggests that both inhibitory and facilitatory serotonergic effects are mediated postsynaptically [1]. The neural sites involved in the mediation of the dual effects of 5HT on receptivity have not been identified. However, previous studies have suggested that P increases 5HT activity in the POA [14,16] and that this effect may be related to the proposed facilitatory effects of 5HT on behavior [1].

The present experiments evaluated 5HT overflow as an index of release in the VMH, MCG, and POA following

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SSDI 0006-8993(95)01403-9

the subcutaneous administration of P in EB-primed rats. Since 5HT has been suggested to have a inhibitory role in the VMH and MCG on sexual behavior [8,20,33], it was hypothesized that the availability of 5HT in the extracellular fluid would decrease following administration of P in a time course consistent with the hormonal effects on either P-dependent activation of proceptivity or the facilitation of receptivity. In the POA, 5HT was monitored to determine if the POA might be a site where P-5HT interactions have facilitatory effects upon behavior.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (180–220 g) were purchased from Charles River (Wilmington, MA) or colony raised in the Southwest Missouri State University animal facility. The rats were housed in wire cages maintained in a reversed, 14L:10D photoperiod (lights off 08:00). Food and water were available ad lib.

The rats were ovariectomized under metofane anesthesia (Pitman-Moore Inc., Mundelein, IL). Subsequent experimental manipulations were initiated 6–10 days following ovariectomy.

2.2. Hormones and treatments

Estradiol benzoate (EB, Sigma Chemical Co., St. Louis) and progesterone (P, Sigma Chemical Co.) were dissolved in sesame oil and propylene glycol, respectively. Rats were primed with 5 μg EB in 0.1 ml vehicle administered subcutaneously (SC) 21–25 h prior to P (0.5 mg/0.1 ml propylene glycol) or vehicle treatment. Progesterone or propylene glycol vehicle were administered SC approximately 3–5 h following probe implantation. The variability in the duration of EB-priming was due to differences in the duration of surgery and in the time required to obtain stable 5HT baseline values following probe implantation.

2.3. Behavior tests

A separate group of rats were tested for the time course of P effects on the facilitation of lordosis and appearance of proceptive behaviors under the gonadal hormone priming regimen utilized in this study. Animals primed with 5 μg EB for 21 h were injected SC with 0.5 mg P or V. Vehicle treated rats were tested once as a time 0 point. Rats treated with P were tested sequentially at 0.5, 1, 1.5, 2, and 3 h after treatment. The vagina was taped to prevent intromission as a means of minimizing the effects of cervical-vaginal stimulation on behavioral responses observed during repeated testing [11]. All behavioral tests were conducted during the dark phase of the photoperiod under illumination provided by red 25 watt bulbs. Each female was placed in a 61 × 31 × 29 cm aquarium containing a Long-Evans male for 20 min or until the female had received 10 mounts. A lordosis quotient (LQ) was calculated by dividing the number of lordosis responses by 10 mounts × 100 [11]. The number of incidences of proceptive responses (ear wiggling and hopping and darting) during the behavior tests were also recorded.

2.4. Serotonin analysis

Serotonin concentrations were analyzed using a laboratory-built HPLC system based on the design by Charles Bradberry et al. [3]. The system maximizes sensitivity by using a pneumatic fluid displacement pump to provide a pulseless flow of mobile phase across the detector cell [3]. The pump consists of a tank of pressurized nitrogen controlled by a regulator. The mobile phase reservoir is a spiral of 0.21 inch i.d. stainless steel tubing with a volume capacity of approximately 350 ml. Samples (10 μl) were introduced to the chromatographic system via a rheodyne injector. Separation was accomplished using a Sepstick 3 μm C-18 microbore column (Bioanalytical Systems). The mobile phase consisted of 100 ml of acetonitrile, 483 mg sodium octanesulfonate, 100 mg EDTA, 150 μl triethylamine, 4.7 g NaH_2PO_4 in 900 ml H_2O. Unadjusted pH was 5.6–5.9. Serotonin was detected electrochemically with a laboratory-built potentiostat and a glassy carbon working electrode (Bioanalytical Systems) maintained at a potential of +0.50 V with respect to a Ag/AgCl reference electrode (Bioanalytical Systems). To minimize electrical noise, the potentiostat was powered by two 12-volt rechargeable gel batteries. The output voltage was recorded as peaks on a Kipp-Zonen strip chart recorder.

2.5. Probe assembly and calibration

Concentric dialysis probes, based on an earlier design (34), were assembled by slipping a 5 mm length of cellulose dialysis fiber (M.W. cutoff 5000, Travenol Laboratories) over the beveled end of a 7 cm length of fused silica tubing (i.d. 101 microns, Polymicro Technologies, Inc.). The membrane-silica assembly was glued into a 5 cm length of 26G stainless steel tubing (Small Parts, Inc.) with 2-ton white epoxy (Devcon) so that approximately 4 mm of membrane was exposed. The exposed cellulose surface was cut to the appropriate length and sealed with 2-ton epoxy. Probe tips used in the POA and VMH were cut to lengths ranging from 1.5 to 2.2 mm. For the MCG, probe tip lengths ranged from 2.4 to 3.0 mm. After the glue dried, a 5 mm length of PE 50 tubing was slipped over both the distal end of the silica fiber and the cannula. A 1 cm length of 26G stainless steel cannula was inserted into the PE 50 sleeve and glued in place with 5-min epoxy (Devcon).

Before implantation, probes were flushed with 70% ethanol [34] and perfused with modified Ringer's solution

(NaCl 147 mM, KCl 2.4 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.0 mM, NaH_2PO_4 0.9 mM, Na_2HPO_4 1.4 mM, pH 7.4; [27]) at a flow rate of 0.75 μl/min. The in vitro recovery was calculated by determining the ratios of 5HT peak heights obtained from the probe with the peak heights measured in an equivalent volume of modified Ringer's solution containing 0.5 to 1×10^{-7} M 5HT at room temperature [38]. Probes exhibiting less than 10% recovery were rejected. Recoveries for most probes ranged from 15–20%.

2.6. Surgical procedures

Surgical implantation of microdialysis probes were performed on rats anesthetized with chloral hydrate (Sigma Chemical Co., 400 mg/kg). Throughout the experiment, additional chloral hydrate was administered as needed to maintain deep anesthesia. Body temperature was maintained with a heating pad. Saline was periodically administered via the tail vein during the course of the experiments. The stereotaxic coordinates for probe implantation, determined from bregma, were: VMH-2.4 mm posterior, 0.7 mm lateral and 9.1 mm below the cortical surface; POA-0.3 mm posterior, 0.6 mm lateral and 9.2 mm below the cortical surface [30]. Modified stereotaxic coordinates for the MCG, determined from lambda, were: 0.3 mm anterior, 2.0 mm lateral, and 6.6 mm below the cortical surface implanted at a 13.9° angle towards the midline [30]. The angle used to implant probes into the MCG served to avoid heavy vascularization present under the suture juncture. The anterior and lateral coordinates represent the central location of access holes drilled with a 1.2 mm i.d. trephine bit. Following removal of the bone plug, the dura was carefully removed under magnification to avoid bleeding. The probe was then slowly lowered into the brain over a 10 to 15 min period. Fig. 1 shows the general probe placement and surface sampled at the respective sites.

2.7. Tetrodotoxin validations

The neuronal origin of 5HT in the perfusates was tested by evaluating the effects of TTX on the basal levels of serotonin for each brain region studied. Tetrodotoxin (TTX, Sigma Chemical Co.) was dissolved in acetate buffer and added as a 1 μM concentration to the modified Ringer's solution. Samples were collected and analyzed until extracellular 5HT levels were stable for 4 consecutive measurements. At this point, the perfusion buffer containing 1 μM TTX was substituted for the standard modified Ringer's solution. Six samples were collected at 20 min intervals afte the introduction of TTX into the perfusion buffer.

2.8. Effect of progesterone on extracellular serotonin levels

P or V were administered SC after the collection of three stable baseline 5HT samples. Following treatment, twelve samples were collected at 20 min intervals. Fig. 2 shows chromatograms of 5HT standards and perfusate peaks from the VMH of rats treated with vehicle (A) or progesterone (B). At the conclusion of the experiments, the rats were given an overdose of chloral hydrate. The brains were preserved in 10% formalin, sectioned and stained with cresyl violet for verification of the implant site.

2.9. Calculations and statistical analysis

Serotonin concentrations were calculated by dividing pg 5HT standard by peak height (pg 5HT/cm peak height)

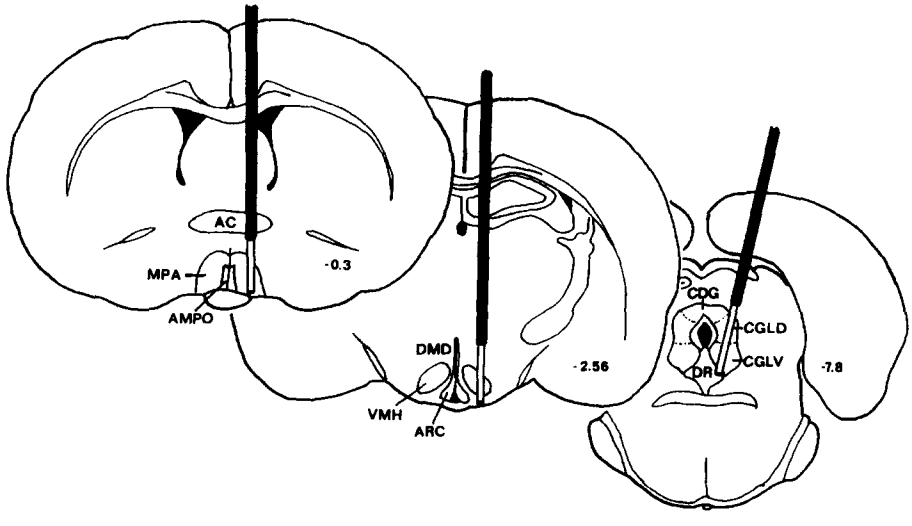

Fig. 1. Coronal sections of the rat brain showing the placement dialysis probes [30]. Anterior commissure (AC), medial preoptic area (MPA), medial preoptic nucleus (AMPO), dorsomedial hypothalamus (DMD), ventromedial hypothalamus (VMH), arcuate nucleus (ARC), dorsal central grey (CDG), dorsal raphe (DR), lateral central grey dorsal (CGLD), lateral central grey ventral (CGLV). Individual sections are numbered to represent distance posterior to bregma.

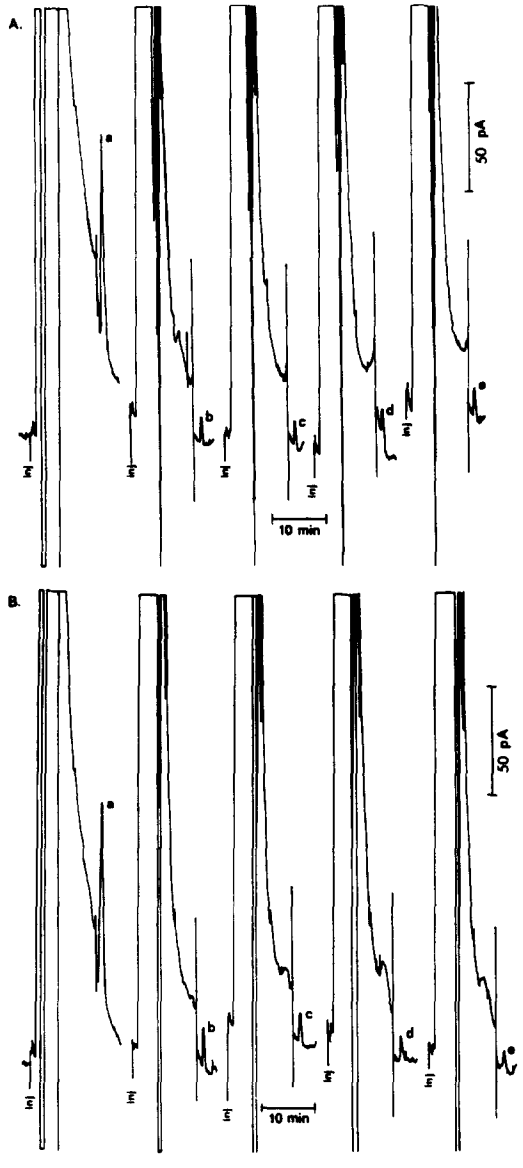

Fig. 2. Representative chromatograms showing the detection of 5HT in perfusates from the VMH from rats treated with vehicle (A) or progesterone (B). In both series of chromatograms (a) represents 2.6 pg of 5HT standard; (b) indicates the peak before treatment; (c) 40 min post-treatment; (d) 80 min post-treatment; (e) 200 min post-treatment.

and multiplying this value by the peak heights obtained from each sample to yield pg 5HT/10 μl sample. There was moderate variation in the basal levels of 5HT when one experiment was compared to another. This was most likely due to slight differences in probe placement and differences in the probe recoveries. To control for this variation all subsequent calculations and statistics were done using the percent of basal serotonin levels. Basal 5HT level (100%) was calculated from the mean of 3 successive samples (4 in the TTX experiments) prior to the initiation of experimental treatments. Serotonin peak heights less than 2:1 signal/noise were considered undetectable and a value based on 2:1 signal-to-noise was used in the calculations.

Results from the group of rats used to determine the time course of P effects on lordosis behavior were analyzed with a one-way anova followed by the Dunnett's test to identify time points that differed from control rats treated with vehicle [42]. The results from the validation studies using TTX were analyzed using a one-way analysis of variance with repeated measures [41]. Comparison of TTX effects to pretreatment samples were done using the Dunnett's test where the sample immediately preceding TTX perfusion served as the control [42]. The effects of P on 5HT overflow were analyzed using a two-way analysis of variance (treatment $\times$ time) with repeated measures where the repeated factor was time [41]. In analyses which revealed a significant time $\times$ treatment interaction, the effects of P on 5HT were compared to V-treated controls using an analysis of simple effects [41]. The effect of treatment over time was compared to pretreatment values using the Dunnett's test where the sample immediately preceding the treatment served as the control value. Significance levels for all statistical tests was set at $P \leq 0.05$.

3. Results

3.1. Time course of progesterone effects on sexual behavior

The time course of P effects on the facilitation of lordosis behavior is summarized in Fig. 3. Under conditions where the priming exposure to EB was 21 h, lordosis quotients were significantly elevated 1.5, 2 and 3 h after P when compared to V-treated controls (Dunnett's, $P < 0.05$). Proceptive responses ($\geq$ 3 responses/test) were only

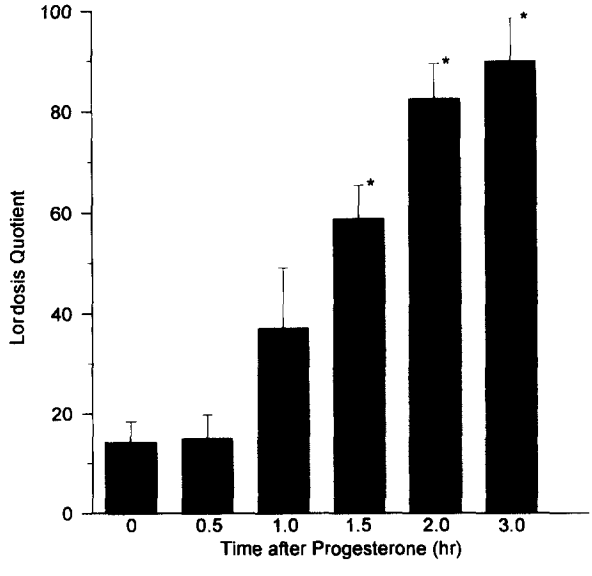

Fig. 3. Time course of progesterone facilitation of lordosis behavior in rats primed with 5 μg EB 21 h earlier. Lordosis quotients were significantly higher than control values 1.5 h after P administration.

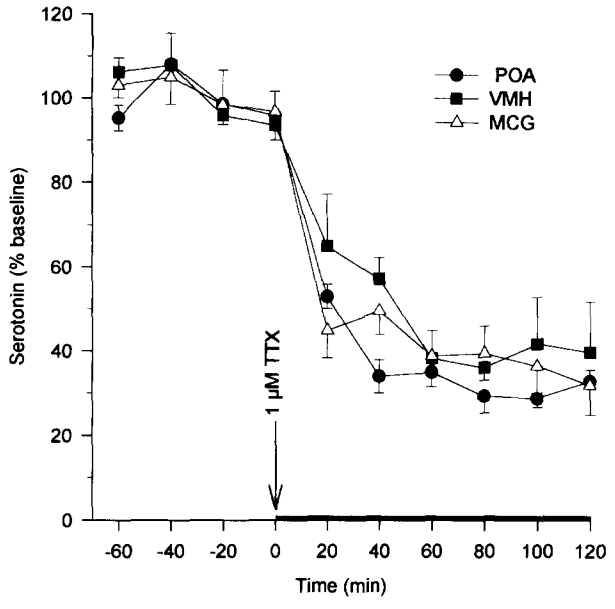

Fig. 4. Effect or TTX on 5HT release in the VMH (squares), POA (circles) and MCG (triangles). Following collection of four consecutive pretreatment samples to establish basal levels of 5HT, standard ACSF was replaced with ACSF containing 1 μM TTX. Values for 5HT are expressed as mean ± S.E.M. percent of the average of the four pretreatment samples. Treatment effects at each time point were compared using one-way analysis of variance with repeated measures. Comparison of TTX effects to pretreatment samples were done using the Dunnett's test where the sample immediately preceding TTX served as control. In all post-treatment samples P was < 0.01.

noted in the tests conducted 2 h (3/8 rats) and 3 h (7/8 rats) after P.

3.2. Validation of neuronal release source

Perfusion with modified Ringer's containing 1 μM TTX resulted in significant reductions in 5HT concentrations in the perfusates from the VMH ($F_{9,45} = 37.9$, $P < 0.0001$). The effect was observed in the first perfusate after TTX (Dunnett's $q_{45,2} = 5.28$, $P < 0.01$) and persisted for the duration of TTX perfusion. TTX perfusion in the VMH reduced extracellular 5HT levels by approximately 65% (Fig. 4). Similarly, TTX application resulted in decreased 5HT concentrations in perfusates from the MCG ($F_{9,45} = 33.01$, $P < 0.0001$). Comparison of the levels of 5HT in the first sample after TTX to the control value was significant (Dunnett's $q_{45,2} = 3.6$, $P < 0.01$). The decrease in 5HT levels in the perfusates was approximately 60% following TTX (Fig. 4). In the POA, TTX significantly decreased 5HT overflow in the first post-treatment sample ($F_{9,54} = 72.99$, $P < 0.0001$; Dunnett's $q_{54,2} = 5.48$, $P < 0.01$) and in all subsequent samples. 5HT overflow in the POA was reduced by approximately 65% of pretreatment values following TTX perfusion (Fig. 4).

3.3. Effects of progesterone on extracellular serotonin levels

Subcutaneous administration of P did not significantly alter 5HT overflow in the POA (Fig. 5). Analysis of variance failed to indicate significant effects of treatment ($F_{1,8} = 2.27$, $P = 0.17$), time ($F_{14,112} = 1.01$, $P = 0.45$) or the treatment × time interaction ($F_{14,112} = 1.09$, $P = 0.38$). Serotonin concentrations in samples collected after P treatment ranged from 85.3–109.2% of the mean of three pretreatment values. In samples collected after V, 5HT values ranged from 83.5–104.4% of the mean of three pretreatment values.

Extracellular 5HT levels rapidly decreased in the MCG following P treatment (Fig. 6). Analysis of variance showed significant effects of treatment ($F_{1,12} = 81.29$, $P < 0.0001$), time ($F_{14,168} = 13.62$, $P < 0.0001$) and treatment × time interaction ($F_{14,168} = 11.59$, $P < 0.0001$). Analysis of simple effects indicated that MCG 5HT levels in rats treated with P were significantly reduced 40 min through 240 min after treatment when compared to V-treated controls ($P < 0.01$). Similarly, the decrease observed following P was significant at 20 min postinjection when compared to preinjection values (Dunnett's $q_{84,4} = 5.14$, $P < 0.01$). At 80 min after P, 5HT levels were reduced to 56 ± 5.1% of pretreatment values. Extracellular 5HT concentrations in the MCG varied little after V treatment.

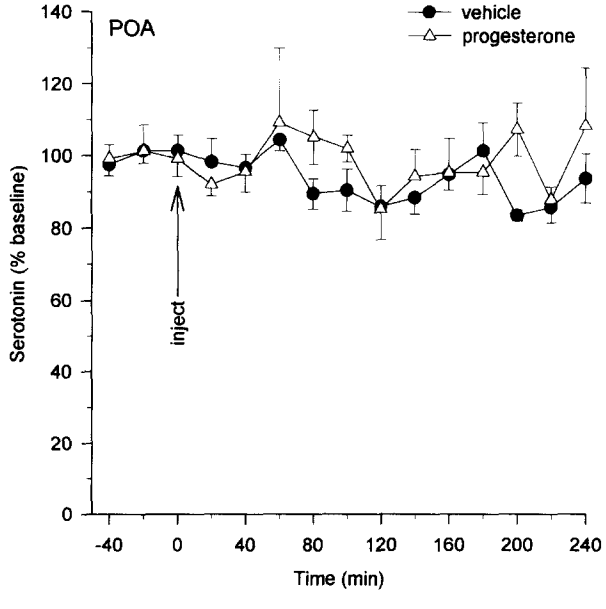

Fig. 5. Effect of P on 5HT release in the POA. Following collection of three consecutive pretreatment samples to establish basal levels of 5HT, progesterone (0.5 mg) or vehicle was injected subcutaneously. Values for 5HT are expressed as mean ± S.E.M. percent of the average of the three pretreatment samples. The results were analyzed using a two-way analysis of variance (treatment × time) with repeated measures where the repeated factor was time.

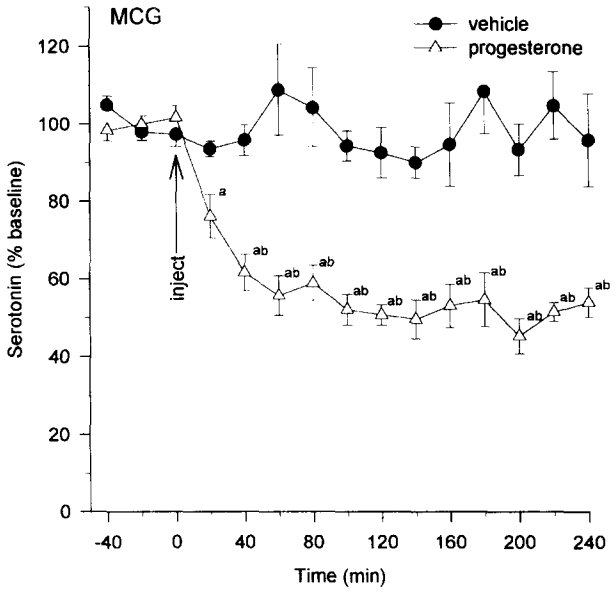

Fig. 6. Effect of P on 5HT release in the MCG. Following collection of three consecutive pretreatment samples to establish basal levels of 5HT, P (0.5 mg) or vehicle was injected subcutaneously. Values for 5HT are expressed as mean ± S.E.M. percent of the average of the three pretreatment samples. The results were analyzed using a two-way analysis of variance (treatment × time) with repeated measures where the repeated factor was time. The effects of P on 5HT were compared to vehicle-treated controls using the analysis of simple effects. The effect of treatment over time was compared to pretreatment values using the Dunnett's test where the sample immediately preceding the treatment served as the control value. [a] $P < 0.01$ when compared to pretreatment values; [b] $P < 0.01$ when compared to vehicle-treated animals.

Serotonin overflow in the VMH was also reduced following P treatment (Fig. 7). Analysis of variance showed significant effects of treatment ($F_{1,12} = 65.62$; $P < 0.0001$), time ($F_{14,168} = 7.0$; $P < 0.0001$) and treatment × time interaction ($F_{14,168} = 7.07$, $P < 0.0001$). A significant effect of P, determined by analysis of simple effects, was first detected 60 min after treatment when compared to control animals ($P < 0.01$). Similarly, by 60 min after P, 5HT levels were decreased when compared to preinjection controls (Dunnett's $q_{84,6} = 2.92$; $P < 0.05$). Serotonin levels decreased to 57 ± 5.9% of pretreatment values 100 min after P. The P-influenced decrease in extracellular 5HT persisted during the remainder of the sampling period with the exception the 3 h time point. In contrast, vehicle treatment had no effect on 5HT overflow when compared to preinjection values.

4. Discussion

The results of the present study demonstrate that systemic administration of P in EB-primed ovariectomized rats decreases extracellular 5HT concentrations in the VMH and MCG but not the POA. The marked decline in extracellular 5HT after TTX perfusion combined with the stable 5HT baseline values obtained in rats treated with vehicle indicate that the decreased 5HT levels measured in the VMH and MCG after P were primarily derived from neuronal release.

Previous studies have shown that systemic administration of P decreased pargyline-induced accumulation of 5HT in pars lateralis of the VMH [16,33] and MCG [33] in ovariectomized rats primed with estrogen. These results were determined using a non-steady state turnover method, in which the administration of pargyline perturbed the system by inhibiting monoamine oxidase [15]. Although this turnover method is believed to provide an estimate of 5HT activity [15], alternative possibilities, such as differences in hormonal effects on monoamine oxidase activity [19], could account for differences in pargyline-induced 5HT accumulation rates. Furthermore, the assumption that the technique actually provides an estimate of release has been questioned since 5HT accumulation rates in animals treated with pargyline do not follow the predicted response following electrical stimulation of the raphe nucleus [37]. The results of the present study, by demonstrating P-dependent decreases in extracellular 5HT in the VMH and

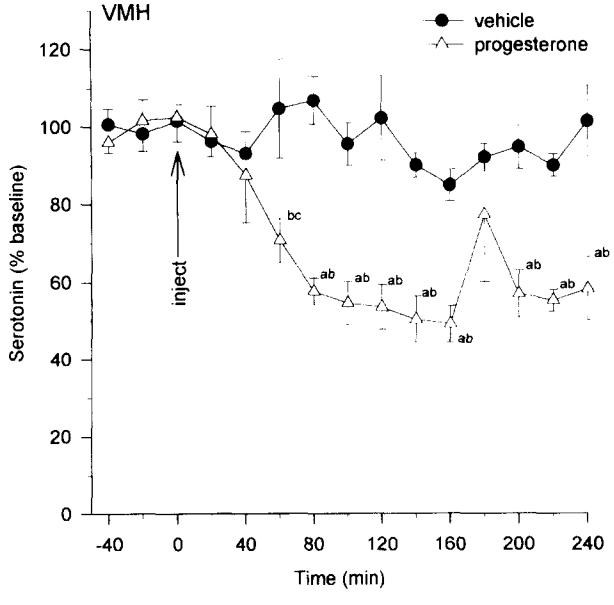

Fig. 7. Effect of P on 5HT release in the VMH. Following collection of three consecutive pretreatment samples to establish basal levels of 5HT, progesterone (0.5 mg) or vehicle was injected subcutaneously. Values for 5HT are expressed as mean ± S.E.M. percent of the average of the three pretreatment samples. The results were analyzed using a two-way analysis of variance (treatment × time) with repeated measures where the repeated factor was time. The effects of P on 5HT were compared to vehicle-treated controls using the analysis of simple effects. The effect of treatment over time was compared to pretreatment values using the Dunnett's test where the sample immediately preceding the treatment served as the control value. [a] $P < 0.01$ when compared to pretreatment values; [b] $P < 0.01$ when compared to vehicle treated animals; [c] $P < 0.05$ when compared to pretreatment values.

MCG in vivo, suggest that the earlier turnover experiments using pargyline provided a valid approach for the general estimation of serotonergic activity [15,16,33] since the primary means by which serotonin concentrations are altered in the extracellular fluid is thought to be through release mechanisms [38].

The time course for the facilitatory effects of P on lordosis behavior and P-dependent effects on proceptive behaviors in the present study was reasonably consistent with earlier studies which used different estrogen-priming conditions and different routes of P administration [9,21]. Although a direct correlation between the changes in extracellular 5HT under current conditions cannot be made with the time course for P effects on behavior; the timing of the P-influenced decline in extracellular 5HT in the VMH is consistent with the duration of P exposure required to facilitate the receptive component of estrous behavior. Within the VMH, extracellular 5HT levels were significantly decreased by 60 min after P treatment and were approximately 60 percent of the pretreatment baseline levels after 80 min in the anesthetized E-primed rat. Similarly, a non-significant increase in lordosis quotients was observable 60 min after P, with significant increases in lordosis quotients evident after a 90 min exposure to P. Supporting the potential relationship between P effects on lordosis and decreases in 5HT in the VMH are numerous demonstrations of behavioral inhibition following intracranial pharmacological enhancements of serotonergic function [2,17,20,40].

Like the VMH, the MCG has long been implicated as an important regulatory site in the control of lordosis behavior [35]. Lesions of the MCG suppress the expression of lordosis [35]. Furthermore, the steroid concentrating neurons in the VMH project to the MCG [4,28] and the projection from the VMH to the MCG must be intact for the expression of behavior [12]. In the MCG, 5HT has also been proposed to play an inhibitory role on lordosis behavior [33]. Progesterone decreases 5HT turnover in the MCG of rats primed with systemic estrogen [33] and intracranial infusions of the $5HT_{1A}$ agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), into the MCG suppresses lordosis [39]. The present results are consistent with earlier turnover work in indicating that one of the actions of P is to decrease release of 5HT in the MCG. However, the time course for the effect does not correspond to the duration of P exposure required for behavioral facilitation. Furthermore, the presence of decreased extracellular 5HT in the MCG 20 min after P administration suggests that P may have acted via a direct mechanism. The effect of P on 5HT turnover in the MCG may depend on hormonal stimulation of the VMH since P-influenced decreases in serotonin turnover were reported in the MCG of rats primed with dilute estrogen cannulae implanted into the VMH [8]. It is possible that the rapid decrease in extracellular 5HT in the MCG resulted from direct effects of P in the VMH. Alternatively, the decrease in 5HT in the MCG may have been due to local non-genomic effects of P in the presence of estrogenic stimulation of the VMH. Previous work in the hamster has demonstrated non-genomic effects of P in the ventral tegmental area which are dependent on genomic steroid effects in the VMH [7]. Finally, it is possible that in the presence of estrogen P may act directly in the MCG to decrease extracellular 5HT. Further studies are necessary to differentiate these possibilities and to determine the relationship of this finding to the expression of lordosis behavior.

The POA has been suggested to play an inhibitory role on female sexual behavior. Lesions of the POA [31,32] or chemical inhibition of protein synthesis facilitated lordosis in steroid-primed rats [22]; while electrical stimulation inhibited behavior [29]. More recently, increases in serotonin activity in medial preoptic nucleus (mPOA) have been proposed to facilitate receptivity [1], based on earlier reports that progesterone increases serotonin turnover in the mPOA [14,16] and the demonstration that 1-(*m*-trifluoromethylphenyl)piperazine, a $5HT_{1A/1B}$ agonist, facilitates behavior in 5,7-dihydroxytryptamine (5,7-DHT) lesioned rats. The results from the present study suggest that P does not affect 5HT release in the POA. Thus, if a serotonergic mechanism regulating lordosis is present in the POA, then it may be mediated via hormonal effects on 5HT receptors rather than activation or inhibition of 5HT terminals. Alternatively, the area sampled by the probe may have been too large to resolve an effect present in the medial preoptic nucleus.

The concept that 5HT exerts a dual role on the regulation of female sexual behavior was proposed based on the demonstration that lordosis could be differentially altered by pharmacological manipulation of 5HT receptor subtypes [13,23]. Subsequent findings suggest that the facilitatory effects of 5HT are mediated by $5HT_2$ receptors while the inhibitory effects involve the activation of $5HT_{1A}$ receptors [10]. More recently, studies utilizing intracranial infusions of the $5HT_{1A}$ agonist, 8-OH-DPAT, have provided evidence suggesting that the inhibitory actions of $5HT_{1A}$ are expressed in the VMH and MCG [39,40]. Furthermore, pharmacological studies in rats lesioned with 5,7-DHT indicate that these effects are mediated postsynaptically in the hypothalamus [1]. Since the administration of gonadal steroids to 5,7-DHT lesioned rats had minimal effects on the density of postsynaptic $5HT_{1A}$ and $5HT_{1B}$ receptors in the VMH, the serotonergic effects on lordosis behavior, whether facilitatory or inhibitory, appear to be regulated by hormonal effects which alter presynaptic function [5]. The present results, by demonstrating a P-influenced decrease in the availability of 5HT in the extracellular fluid in the VMH and MCG suggest that the most likely mechanism for disinhibiting $5HT_{1A}$ receptors in these sites involves P-mediated effects which result in the inhibition of serotonin release. The site(s) where gonadal steroids act to exert the facilitatory component of 5HT action remain elusive.

Acknowledgements

Support for these studies from NSF grant IBN-9596009 to K.J.R. is gratefully acknowledged. We would also like to thank Drs. Charles Bradberry and Bita Moghaddam for their help in developing the microdialysis instrumentation and procedure and Dr. Victoria Luine for helpful discussions and for critically reading this manuscript.

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