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Delta opioid modulation of hypothalamic serotonergic neurotransmission in the ovariectomized and steroid-primed rat

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Abstract

OBJECTIVES: We have investigated the modulating effects of DPDPE (a δ opioid agonist) and opioid receptor antagonists on both LH release and indoleamine concentrations in specific hypothalamic regions of the ovx and steroid-primed rat.

METHODS: DPDPE was intracerebroventricularly infused alone or with either ICI 154129 (a δ opioid antagonist) or naloxone under ketamine anesthesia. Blood samples were collected at hourly intervals on the afternoon of the anticipated LH surge. The rats were then decapitated and the medial preoptic area (MPOA), suprachiasmatic nucleus (SCN), median eminence (ME) and arcuate nucleus (ARN) surgically isolated by micropunch. Concentrations of 5-HT and its metabolite (5-HIAA) in these samples were determined by HPLC with ECD. Plasma LH levels were measured by RIA.

RESULTS: The δ-agonist significantly reduced 5-HT concentrations in the SCN, ME and ARN, but not in the MPOA. 5-HIAA levels were decreased, but these changes were significant in only the MPOA and ARN compared to the control group. ICI 154129 had no significant effects on 5-HT release and turnover in any of the hypothalamic regions examined. However, co-administration of DPDPE with naloxone resulted in significant increases in 5-HT and 5-HIAA concentrations in the MPOA, SCN, ME and ARN compared to the DPDPE-treated group. Plasma LH levels were either low or undetectable in all groups.

CONCLUSIONS: The present results suggest that δ-opioid receptors are involved in the opioid inhibition of the serotonergic neurotransmission in the hypothalamus. It is thought that the ketamine anesthesia interfered with LH secretory systems.

ORIGINAL ARTICLE

Introduction

A multiplicity of neurotransmitter systems appear to be involved in the central control of gonadotrophin-releasing hormone (GnRH) activity and hence luteinising hormone (LH) release [1, 2]. The opioid system exerts a physiological tonic inhibitory effect on GnRH neurons. Enhancement of LH release following administration of naloxone (an opioid antagonist) has been shown [3]. Conversely, administration of opioid agonists, just before the critical period on the day of pro-estrus, inhibits release of the preovulatory LH surge and hence ovulation [4]. It has been proposed that a reduction in the endogenous opioid tone amplifies the anticipated LH surge in oestogen-treated ovariectomized (ovx) rats [5, 6]. Activation of δ-opioid receptors has been shown to reduce naloxone-induced LH release, suggesting a possible role of δ-receptors in opioidergic modulation of LH secretion in women [7]. Existence of three major classes of opioid receptor subtypes (μ, κ and δ) has been shown in the hypothalamus as well as in other brain areas [8, 9].

Direct synaptic connections exist between opioid peptidergic nerve terminals and GnRH neurons in the medial preoptic area (MPOA) and median eminence (ME) [10]. There is some evidence for direct opioidergic modulation of GnRH release [11]. However, a bulk of evidence has indicated that the opioids may act indirectly by influencing the brain monoaminergic systems. The hypothalamus possesses serotonergic fibers which arise from neuronal cell bodies located in the raphe nuclei and have synapse on GnRH-secreting neurons [12]. Direct stimulation of GnRH neurons by 5-hydroxytryptamine (5-HT, serotonin) has been shown *in vitro* [13]. Opioid binding sites on serotonergic neurons have been reported [14]. It has been suggested that δ-opioid receptors may modulate serotonergic neurons in the brainstem [15].

In the present study, we have investigated the modulating effects of DPDPE (a δ opioid agonist) either alone or when co-administered with opioid receptor antagonists (ICI 154,129 and naloxone) on both LH release and indolamine concentrations in specific hypothalamic regions in the ovx and steroid-primed rat.

Material and methods

Female Sprague-Dawley rats (Harlan UK Ltd., Oxon, England) weighing 250–270g were maintained under controlled temperature (21±1°C) and light conditions (lights on from 07. 00h to 19.00h). Food and water were provided *ad libitum*. The animals were ovx under halothane anesthesia and then allowed to recover for a period of two weeks. Forty-eight hours prior to experimentation the rats were injected subcutaneously (sc) with 17β-estradiol (5μg/0.2 ml olive oil). Progesterone (0.5mg/0.2 ml olive oil) was administered sc four hours before the experiments were carried out.

On the afternoon of the anticipated LH surge, the animals were anesthetized with ketamine hydrochloride (Parke-Davis, Gwent, UK). Surgical anesthesia was maintained by further periodic intramuscular injections of the anesthetic. A heparinized cannula was inserted into the right femoral artery. The animals were then mounted on a stereotaxic apparatus and intracerebroventricularly (icv) infused with either DPDPE (δ-agonist; 50μg/10μl; n=8), (predominantly μ-antagonist), DPDPE plus ICI 154,129 (δ-antagonist; 60μg/10μl; n=7) or DPDPE plus naloxone (60μg/10μl; n=7) at 14.00h on the afternoon of the anticipated LH surge. Controls were infused with 10μl sterile saline alone. Blood samples (200μl) were collected via the indwelling cannula at hourly intervals from the anesthetized animals commencing at 14.00h. At 19.00h the rats were decapitated, and the brains rapidly removed and frozen on dry ice. 500 μm coronal brain slices were cut. The MPOA, suprachiasmatic nucleus (SCN), ME and ARN were micropunched according to the Stereotaxic Atlas of the Rat Brain [16].

Naloxone and ICI 154,129 were purchased from Sigma Chemicals Corporation (Poole, Dorset, UK) and Cambridge Research Biochemicals Ltd (Cheshire, UK), respectively. DPDPE was generously provided by the National Institute on Drug Abuse (Rockville, MD, USA).

The experimental procedures detailed in our project and personal licenses were approved by the Home Office (U.K.) Animal Research Ethics Committee.

Indolamine Measurement: Specific hypothalamic areas collected were stored at –80°C prior to analysis. 100μl of 0.1M HCl was added to the samples, along with another 50μl of HCl containing 2ng of 3,4-dihydroxybenzylamine as an internal standard. The samples were homogenized and then centrifuged (3000rpm, 4°C, 10 mins). Aliquots (10μl) of supernatant were injected on to a reverse phase high performance liquid chromatographic (HPLC) column (S5ODS2-250A, 5μm, 4.6 mm i.d.x25 cm) coupled to an electrochemical detector (ECD). Indolamine concentrations of the four hypothalamic regions were simultaneously detected. The method has been described in our previous report [17]. The minimum detectable level of amines was 0.4–0.5 pg/μg protein.

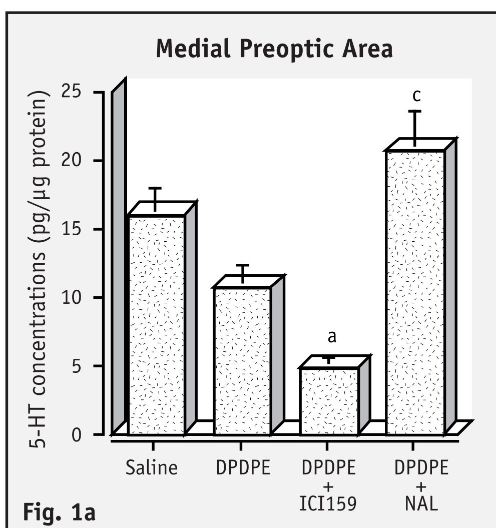
LH Assay: Plasma LH levels were measured by radioimmunoassay (RIA). The immunoreactivity of LH was used to determine its relative abundance in each of the serum samples using a homologous double antibody. RIA reagents were obtained from the National Hormone and Pituitary Program (Baltimore, MD, USA). The inter- and intra-assay co-efficients of variation were 8.0% and 9.5%, respectively. Sensitivity of the assay was 10 pg/tube (1ng/ml).

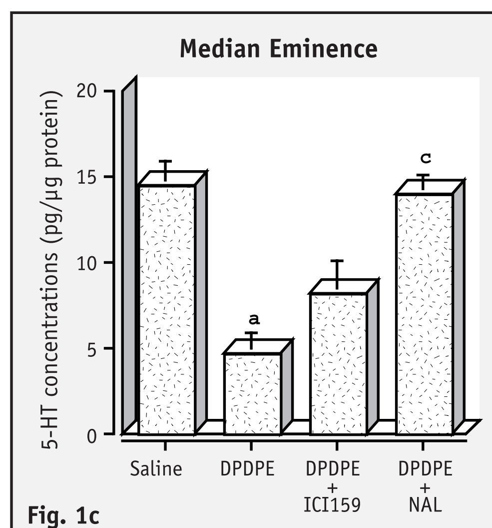
Protein Estimation: The protein content of the tissue pellet remaining after HPLC-ECD was determined from its absorbance at 595 nm after reaction with Coomassie Blue G250 (Pierce, Luton, UK). The weight of protein was calculated from a standard curve prepared using bovine serum albumin as detailed before [17].

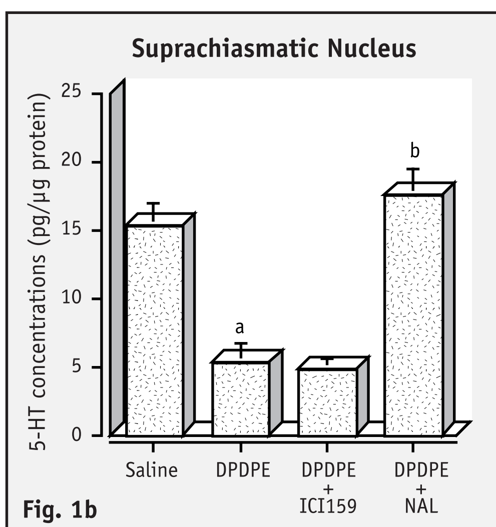
Statistics: One-way analysis of variance was performed on the data. When the F-test was significant it was followed by the Kruskal-Wallis non-parametric test in groups where standard deviations were large between means.

Results

Indolamine findings: 5-HT and 5-hydroxyindole acetic acid (5-HIAA) results are shown in Figures 1 and 2. DPDPE administration significantly reduced 5-HT concentrations in the SCN, ME and ARN, but not in the MPOA, compared to the control group values. When DPDPE was co-administered with the δ-opioid receptor antagonist, 5-HT concentrations remained unaffected in the SCN, ME







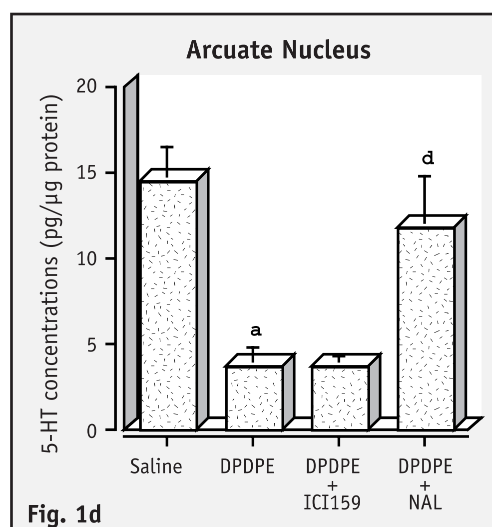
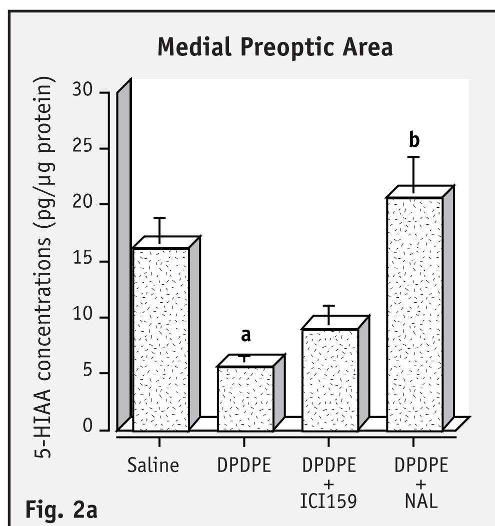
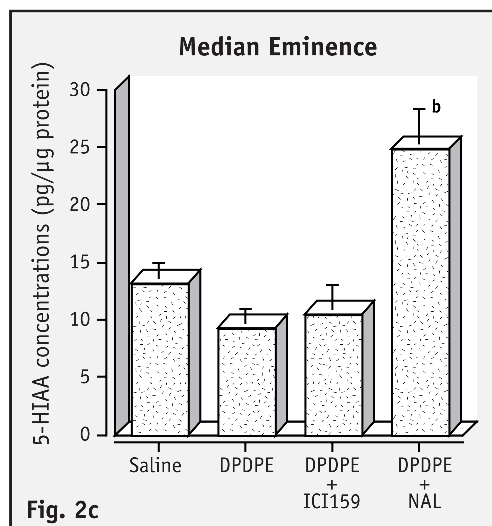
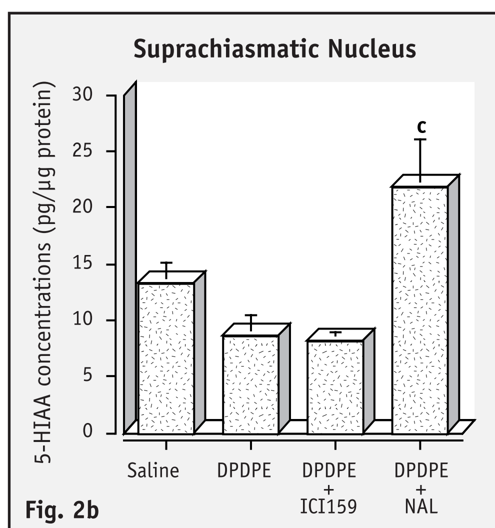


Figure 1 (a, b, c, d). 5-hydroxytryptamine concentrations (pg/µg protein±SEM) in the MPOA, SCN, ME and ARN of the rat hypothalamus at 19.00h on the afternoon of the anticipated LH surge following administration or co-administration of saline and δ-opioid agonist (DPDPE) and antagonists (ICI 154,129 and naloxone) at 14.00h on the same day. **a:** $p<0.001$ compared to the saline-treated animals, **b:** $p<0.01$, **c:** $p<0.001$, **d:** $p<0.05$ compared to the DPDPE-treated animals, using One-way ANOVA and the Kruskal-Wallis non-parametric test.







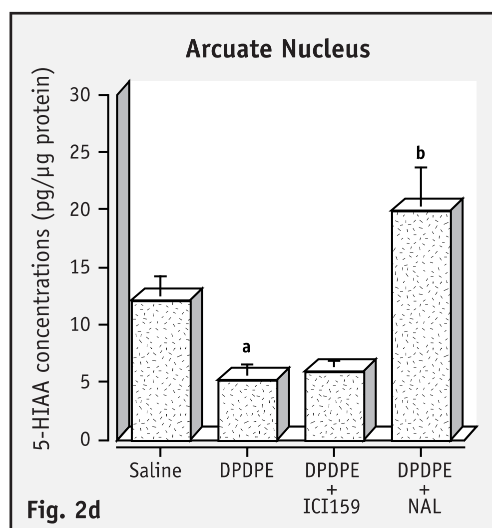


Figure 2 (a, b, c, d). 5-hydroxyindole acetic acid concentrations (pg/μg protein±SEM) in the MPOA, SCN, ME and ARN of the rat hypothalamus at 19.00h on the afternoon of the anticipated LH surge following administration or co-administration of saline and δ-opioid agonist (DPDPE) and antagonists (ICI 154,129 and naloxone) at 14.00h on the same day. **a**: p<0.005 compared to the saline-treated animals, **b**: p<0.001, **c**: p<0.05 compared to the DPDPE-treated animals, using One-way ANOVA and the Kruskal-Wallis non-parametric test.

and ARN, but were significantly decreased in the MPOA compared to the DPDPE-treated animals. Co-administration of DPDPE with naloxone significantly increased the concentrations of 5-HT in all hypothalamic areas examined in comparison to DPDPE-treated animals.

Administration of the δ-opioid agonist resulted in significant decreases in the 5-HIAA levels in the MPOA and ARN compared to the control group. However, DPDPE had no significant effect on the 5-HIAA concentrations in the SCN and ME. Co-administration of DPDPE and ICI 154,129 caused no significant changes of 5-HIAA concentrations in any of the hypothalamic regions examined. 5-HIAA levels were significantly increased following the co-administration of DPDPE with naloxone in the MPOA, SCN, ME and ARN compared to the DPDPE-treated animals.

LH findings: In the ketamine-anesthetized control animals, there were some rises in plasma LH levels over the afternoon sampling intervals (in seven out of 15). Plasma LH levels in these seven animals at hourly sampling intervals were as follows: 15.00h: 1.8±0.39; 16.00h: 2.1±0.49; 17.00h: 3.6±1.22; 18.00h: 3.3±0.8 and 19.00h: 3.4±0.65. However, in the remainder of the controls and also in the DPDPE, ICI 154,129 and naloxone-treated rats, LH levels were consistently either low or below the limit of detection. In the control animals, concentrations of 5-HT and 5-HIAA in all four hypothalamic areas were not significantly different between the subgroups with respect to the plasma LH levels detected.

Discussion

It has been suggested that endogenous opioid peptides alter the central serotonergic neurotransmission by their action at pre-synaptic nerve terminals [18, 19]. Opioid receptors modulate the physiological release of 5-HT from serotonergic neurons in the rat brain [20]. Presence of δ-opioid receptors has been shown in the raphe nucleus and in the hypothalamus [9]. In the present study, 5-HT concentrations in all four hypothalamic regions examined (except in the MPOA) were significantly reduced following icv administration of DPDPE. The δ-opioid agonist reduced 5-HIAA levels in all four hypothalamic areas, but these decreases were found to be significant in only the MPOA and ARN. These results thus indicate that opioid inhibition of the serotonergic system within the hypothalamus may be exerted through δ-opioid receptors in addition to well documented actions of μ- and κ- types. It appears that the δ-opioid agonist lowers 5-HT synthesis by inhibiting its release from the nerve terminals as both 5-HT and 5-HIAA levels were reduced in parallel.

The specificity of δ-opioid modulation of the serotonergic neuronal activity was tested by icv co-administration of DPDPE with ICI 154,129. Surprisingly, the δ-opioid antagonist, ICI 154,129, failed to prevent the suppressive actions of DPDPE on the hypothalamic 5-HT and 5-HIAA concentrations. However, an enhanced serotonergic activity was observed after icv infusion of the δ-agonist concomitantly with naloxone (a primarily μ antagonist) in the MPOA, SCN, ME and ARN. There have been reports of cross-communication between the δ- and μ-opioid receptor subtypes in the brain [21]. In the rat brain, μ- and δ-receptors can exist on the same neuron and are physically associated [22]. It is not inconceivable that the activation of the δ-receptors would allosterically influence μ-receptor activity [23]. Therefore, it is postulated that DPDPE may influence 5-HT release and turnover primarily by acting at μ receptors. However, this hypothesis remains to be further investigated.

A role for the involvement of the serotonergic system in the central control of gonadotrophin secretion has been suggested [24, 25]. It has been proposed that the serotonergic system changes during sexual maturation from a stimulatory effect in prepubertal to an inhibitory action in adult rats on gonadotropin secretion [26]. Pharmacologically, administration of a 5-HT agonist attenuated the proestrous surge of LH [27]. An increase in the density of 5-HT2A receptors in the forebrain has been reported at pro-estrus in intact female rats [28]. Furthermore, 5-HT2A receptors play an important role in the estradiol-induced surge of GnRH in the rat [29]. It has also been suggested that dorsal raphe nucleus-ME 5-HT projections may exert a facilitatory influence while medial raphe nucleus-MPOA is inhibitory to the LH surge [30]. In the present study, plasma LH levels were found to be either low or undetectable in all sampling intervals following the administration of DPDPE or its co-administration with ICI 154129 or naloxone on the afternoon of the anticipated LH surge. Control group values were also low and inconsistent. It is therefore difficult to draw any conclusions regarding interaction between the δ-opioid receptors and serotonergic systems in controlling LH secretion.

It is suspected that ketamine anesthesia markedly suppressed the anticipated LH surge in all experimental groups in our study. In contrast to the present findings, it has previously been claimed that in ketamine-anesthetized rats, peak LH responses are not significantly different from those in unanesthetized control animals [31]. Ketamine anesthesia did not seem to alter hypothalamic 5-HT release and/or turnover. It might reduce responsiveness of the gonadotrops to the surge of GnRH [32]. However, the mechanisms by which ketamine produces such effects are currently unknown.

In conclusion, the present results indicate that δ-opioid receptors may be involved in the opioid inhibition of the serotonergic neurotransmission in the hypothalamus during the anticipated LH surge in the rat. It is suggested that anesthetics should be avoided when studying the neuroendocrine control of LH secretion, if at all possible.

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