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Effects of morphine on prolactin receptors in the rat brain

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Abstract

The effect of chronically given morphine on the binding of ovine prolactin (oPRL) to specific areas in the male rat brain was studied. The drug was delivered through subcutaneously implanted miniosmotic pumps. The results indicated that the density of prolactin binding sites in the hypothalamus and the choroid plexus was significantly decreased in the acute phase of morphine administration but restored to control levels when tolerance to morphine was developed. The decrease in prolactin binding was contrasted by elevated plasma levels of the hormone. A negative correlation was found between the hormone concentration in plasma and the density of its binding sites in the hypothalamus and choroid plexus. The hormone-binding sites in these two regions were further characterized with regard to binding constants and molecular sizes. The relevance of the present results with respect to the hypothalamic control of prolactin secretion is discussed.

Key words: Prolactin; Receptor; Rat brain; Morphine; Dependence

1. Introduction

The acute administration of opioid agonists elicits changes in the release of anterior pituitary hormones. Plasma levels of e.g. prolactin (PRL) and growth hormone (GH) are seen to increase in rodents, in non-human primates as well as in humans following acute opioid administrations [1–7]. Both morphine and β-endorphin, when injected (i.v.) in adult male rhesus monkeys, were found to produce immediate increases in PRL, which remained elevated for 3 h [8]. The stimulatory effect of morphine was reversed by administration of the primarily μ-opioid receptor antagonist naloxone. In male rats, intraventricular injections of morphine resulted in a significant increase of plasma PRL after 10 min [9]. Studies have also shown that administration of morphine in the rat at a high dose (15 mg/kg s.c.) produced at 4 h after administration suppressed plasma PRL concentrations [10]. The authors concluded that morphine has a biphasic effect on the prolactin secretion.

The neuroendocrine regulation of the PRL secretion is known to be a multi-factorial process, but dopamine (DA) secreted by the tuberoinfundibular dopaminergic neurons of the hypothalamus is believed to exert a predominant tonic inhibitory control on the secretion of the hormone from the pituitary . In the hypothalamus PRL receptors may mediate an action of the hormone on the DA-turnover [11,12]. It has also been shown that opioid agonists such as morphine or Met-enkephalin stimulate a naloxone-reversible PRL secretion from isolated adenohypophysial cells when they are co-incubated with hypothalamic fragments [13]. These findings support the hypothesis that one site of action of opioid compounds on pituitary PRL secretion is at the hypothalamic level . However, there are still several questions to be addressed concerning the molecular mechanisms through which the opioids exert their effects and through which the hormone release is attenuated [10] following pretreatment or chronic treatment with morphine.

In this work we have directed studies on prolactin-binding in the male rat brain following chronic morphine administration. The drug was delivered by subcutaneously implanted miniosmotic pumps and the PRL-binding in different specific brain regions (including hypothalamus, striatum, cortex and choroid plexus) was determined at different stages of morphine dependence. The putative prolactin receptors were characterized with regard to binding constants and molecular sizes.

2. Materials and methods

2.1. Materials

Male Sprague–Dawley (Alab, Sweden) rats weighing 240–260 g were housed in a room with controlled humidity (60%) and temperature

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(23°C) and with fixed lighting schedule (light on from 8.00 h to 20.00 h). Water and food were given ad libitum. The rats were adapted to the environment at least one week before the start of the experiment. One day before implantation of the pumps the animals were placed in individual cages with transparent walls and were kept there until the ending of the experiment.

All rats were randomly divided into four groups each consisting of 6 experimental and 6 control animals. Mini-osmotic pumps (Azlet, 2ML) were filled with morphine acetate (70 mg/ml) or saline and were implanted subcutaneously on the back under diethyl ether anaesthesia [14,15]. Experimental animals received morphine during varying time intervals. The first group (acute phase- or AP-group) was decapitated after 24 h following pump implantation. The second group (tolerance- or T-group) was decapitated after 120 h, just before pump removal. The third group (withdrawal- or W-group) was decapitated 20 hours after the pumps were removed (140 h after implantation). The fourth group (abstinence- or A- group) was decapitated 48 h after the pump removal or 168 h after the implantation. The development of tolerance and dependence was checked by determination of body weight, water consumption and pain sensitivity as described elsewhere [14,15].

2.2. Tissue dissection

Following decapitation brain and liver were immediately removed and placed on ice. The brains were dissected on ice using a rat brain matrix (Activational Systems Inc., Morterra Drive Warren, Michigan, USA) as described previously [15]. The tissues collected were kept frozen at −80 °C until further processing.

2.3. Preparation of receptor fractions

The individual tissues were homogenized by ultrasonification for 5 sec at 0 °C in 25 mM Tris-HCl buffer pH 7.4, containing sucrose (10%, w/v). The volumes were for choroid plexus and hypothalamus: 1 ml/20 mg; for cortex, hippocampus and striatum : 2 ml/100 mg; and for liver: 5 ml/g. The homogenates were centrifuged at 9,000 × *g* for 10 min and the supernatants were recentrifuged at 40,000 × *g* for 60 min at 4°C. The pellet fractions were collected and suspended in 25 mM Tris-HCl buffer, pH 7.4. All procedures were performed at 5°C or on ice and the suspensions were used immediately or stored at −80°C.

2.4. Hormone labelling

A highly purified preparation of oPRL (molecular mass (M_r) 23,000, Sigma Chemical Company, St. Louis, MO, USA) was labelled with ^{125}I by the Chloramine T method. The specific activity of the labelled hormone was 145–160 μCi/μg.

2.5. Binding assays

The assay for prolactin-binding to the receptor homogenates was conducted as described in a preceding paper [16]. Association constants and binding capacities were calculated [17] using the computer program LIGAND (Biosoft, Cambridge, UK). The analyses were based on experiments wherein [^{125}I]oPRL was displaced by different amounts of unlabelled oPRL. Protein concentrations were determined according to Lowry et al. [18].

2.6. Radioimmunoassay

Plasma PRL was determined by radioimmunoassays (RIA) according to the procedure by Neill and Reichert [19] using a kit supplied by AIP (Amersham, UK)

2.7. Covalent cross-linking of receptors to [^{125}I]oPRL

Cross-linking of the iodinated hormone to receptor membranes was performed using disuccinimidyl subcrate (DSS, ICN Biomedicals, Planview, NY, USA). Receptor homogenates (100 μl) were incubated overnight with [^{125}I]oPRL (60,000 cpm) at room temperature in a total volume of 250 μl PBS containing $MgCl_2$ (10 mM). Subsequently, 50 μl of DSS (2.5 mM , freshly dissolved in dimethyl sulfoxide) was added and the tubes were kept at 0° C for 15 min. The reaction was terminated by the addition of 15 μl 2M Tris-HCl buffer, pH 7.4, containing 1 mM EDTA. Samples with an about 200-fold excess of unlabelled hormone were used as controls and run in parallel.

2.8. SDS-electrtrophoresis of cross-linked complexes

Following cross-linking, electrophoresis was performed in a discontinuous polyacrylamide gel as described in a preceding paper [16]. The samples (315 μl) were mixed with a 'sample cocktail' (75 μl of 0.3 M Tris-HCl buffer pH 8.8 , containing 14% (w/v) sodium dodecyl sulphate (SDS), 10 mM dithiothreitol, 7.5 μl of 50% Trasylol (Fluka Chemika A.G., Switzerland), and 36% (w/v) sucrose) and boiled for 5 min. After alkylation with 60 mM iodoacetamide for 15 min at 24°C, aliquots of 40 μl were electrophoresed for 45 min at 200 V using a Mini-Protean II apparatus (Bio-Rad Laboratories, Richmond, CA, USA). To locate the molecular weight markers the gels were stained with Coomassie brilliant blue R prior to autoradiography (Hypercassette, Amersham, UK).

2.9. Statistical analysis

The statistical analysis of data was performed by parametric or non-parametric one-way ANOVA and by using Student's unpaired *t*-test. All calculations were carried out with the 'Logstat' program.

3. Results

Maximal analgetic effect of morphine was observed 24 h following pump implantation. Analgesia was attenuated after additional 24 h in spite of continuous delivery of morphine, that indicated the development of tolerance. The sensitivity of experimental animals to pain remained at control levels until the pump was removed after 120 h . Twenty hours after removal of the pump, we observed the maximal expression of withdrawal signs (decreased body weight, decreased water consumption and hyperalgesia), which completely disappeared 48 h after pump removal, the abstinence state. All these observations were thus in accordance with those described for this experimental animal model in a preceding paper [15].

The binding of oPRL to choroid plexus and hypothalamus was significantly reduced in morphine treated rats 24 h after pump implantation (Table. 1, AP), i.e. in the acute phase. Following development of morphine tolerance (Table.1, T) the density of PRL-binding was restored to control values in both tissues. During the state of withdrawal (W) an increase in PRL-binding was found in the hypothalamus, whereas a decrease was seen in the choroid plexus during the abstinence phase (A). No significant alteration was observed (Table 1) in the other tissues at the times studied.

Data obtained by radioimmunoassays indicated a significant increase in plasma PRL at 24 h following pump implantation (morphine: 6.8 ± 1.0 ng/ml, control: 3.4 ± 0.45 ng/ml; $n = 6$, $P < 0.05$). Furthermore, a negative correlation was found between the plasma levels of the hormone and the density of its binding sites in the choroid plexus and the hypothalamus (Fig. 1).

In order to exclude that the decreased binding simply resulted from masked receptors formed by elevated prolactin levels, additional experiments were performed. Thus, prior to binding experiments, the receptor preparations were desaturated with 4 M $MgCl_2$ according to Kelly et al. [20]. The result indicated that this treatment slightly increased the binding in the liver but the effect on the brain tissue was negligible. The significant reduction in the PRL binding observed in choroid plexus and

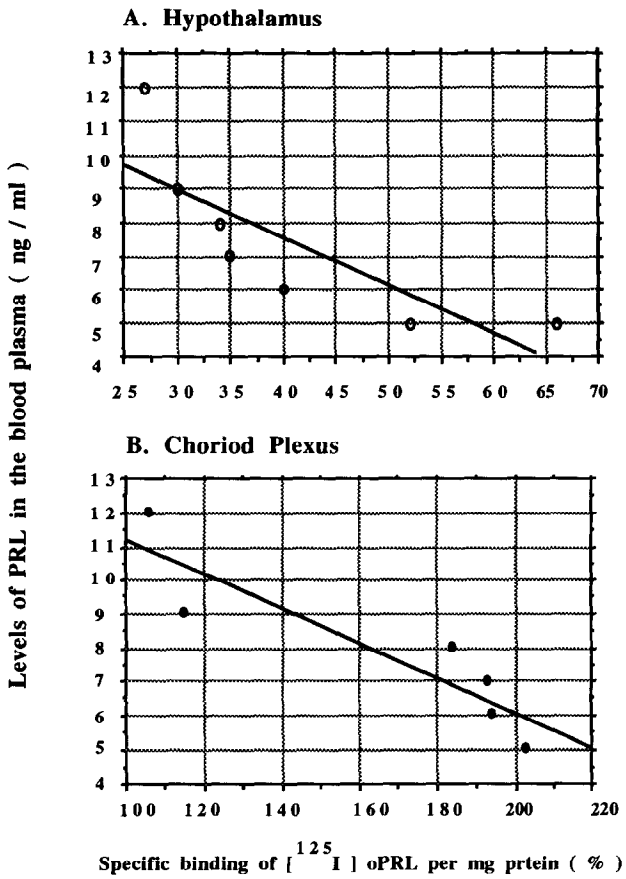

Fig. 1. Plasma levels of rat PRL plotted versus density of PRL binding sites in (A) hypothalamus and (B) choroid plexus from morphine treated rats. Tissues and plasma samples were collected at 24 h following pump implantation. The correlation between the hormone level and the specific PRL binding is significantly negative (A, plasma-hypothalamus: $y = -0.15x + 13.4$, $r^2 = 0.658$, $P < 0.05$; B, plasma-choroid plexus: $y = -0.52x + 16.4$, $r^2 = 0.812$, $P < 0.05$).

hypothalamus from morphine treated animals at 24 h (Table 1) remained also in receptor preparations desaturated with $MgCl_2$.

The most pronounced alteration in prolactin binding was thus observed in choroid plexus and hypothalamus 24 h after implantation of the morphine pumps. These tissues were also found to exhibit the highest content of PRL-binding sites (Table 1). To further characterize the nature of these changes individual tissue samples collected from hypothalamus and choroid plexus 24 h after pump implantation (AP-group) were pooled. The binding sites in both hypothalamus and choroid plexus were found to be saturable as well as dependent on pH and temperature. Calculation of affinity constants yielded values within the nanomolar range (Table 2), which did not differ in control and experimental animals, whereas the binding capacities were significantly lower for tissue pools obtained from morphine treated animals than for pools from control animals.

The results of analytical electrophoresis of cross-linked hormone-receptor complexes (receptor material originating from choroid plexus and hypothalamus) indicated the presence of only one specific binding entity for prolactin (Fig. 2). The complex from each tissue was visualized as a single band corresponding to a protein of M_r 59,000 (choroid plexus) and 60,000 (hypothalamus). After subtraction of M_r 23,000 for oPRL these values are consistent with M_r values of 36,000 and 37,000 for the respective binding unit.

It could further be seen in Fig. 2 that the density of the bands corresponding to hormone–receptor complexes obtained from morphine-treated animals (AP-group) were significantly reduced (lanes C and G), which is in accordance with the result shown in Table 1.

4. Discussion

In this study we have examined prolactin sites in different regions of rat brain and liver following exposure to morphine. Only a few studies dealing with PRL binding to discrete areas in the rat brain have been published (e.g. [21]). To our knowledge this is the first study report-

Table 1
Specific binding of [^{125}I]-oPRL to membranes from rat brain and liver

Tissue	Specific binding of ^{125}I-oPRL per mg protein (%)							
	AP (group)		T (group)		W (group)		A (group)	
	C	M	C	M	C	M	C	M
Choroid plexus	228 ± 31	123 ± 14**	217 ± 20	200 ± 79	206 ± 6	210 ± 42	227 ± 23	159 ± 26*
Cortex	5.5 ± 1.9	3.8 ± 2.3	5.4 ± 1.8	4.74 ± 2.8	5.3 ± 1.2	5.9 ± 2.3	5.5 ± 1.8	3.9 ± 2.0
Hippocampus	12.3 ± 2.5	10.4 ± 2.6	12.1 ± 2.5	12.3 ± 3.1	12.7 ± 6	14.2 ± 6.8	12.2 ± 5.4	9.9 ± 3.3
Hypothalamus	69.3 ± 3.9	32 ± 13**	65 ± 11.2	62 ± 9.5	67 ± 1.0	81 ± 2.6*	62 ± 8.9	61 ± 8.0
Striatum	10.3 ± 4.1	5.1 ± 2.2	11.0 ± 5.1	10.1 ± 8.6	9.7 ± 2.4	7.5 ± 3.2	9.7 ± 4.5	7.4 ± 2.0
Liver	11.3 ± 3.0	13.0 ± 2.6	10.5 ± 2.0	9.6 ± 1.7	10.2 ± 4.1	7.7 ± 3.0	10.8 ± 2.6	7.5 ± 3.4

The values are means ± S.D. ($n = 6$). AP, Acute phase; T, Tolerance; W, Withdrawal; A, Abstinence; C, Control; M, Morphine. Statiscal evaluation was done with ANOVA (analysis of variance) and Student's unpaired t-test (*$P < 0.05$, **$P < 0.01$). The alterations seen in choroid plexus and hypothalamus were significant both with ANOVA and Student's t-test. The protein concentrations were for Choroid Plexus: 0.748 (mg/ml); for Cortex: 8.49 (mg/ml); for Hippocampus: 3.57 (mg/ml); for Hypothalamus: 2.02 (mg/ml); for Striatum: 2.79 (mg/ml); and for Liver: 13.8 (mg/ml).

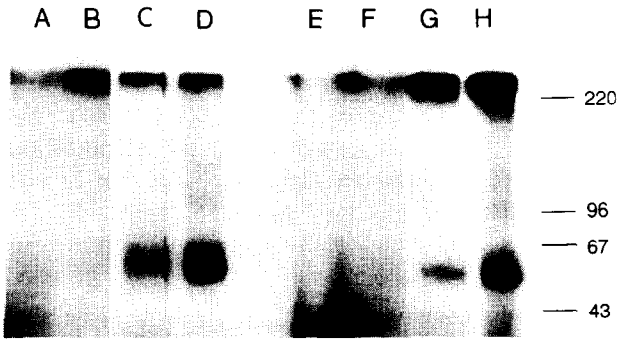

Fig. 2. SDS-electrophoresis of cross-linked hormone–receptor complexes. Membrane-bound receptors from rat hypothalamus (A–D) and choroid plexus (E–H) were incubated and subsequently covalently cross-linked to [^{125}I]oPRL (70,000 cpm) by disuccinimidyl suberate and aliquots of the complexes formed were subjected to SDS electrophoresis and autoradiography. Receptors from control groups (lanes D and H) indicate marked bands, whereas those from the acute phase of morphine treatment (C and G) appear as very weak bands. Samples applied in lanes A, B and E, F were prior to cross-linking incubated both with labelled and an excess of unlabelled hormone. The positions of molecular weight markers (molecular mass $\times 10^{-3}$) are indicated in the right margin.

ing binding constants and molecular sizes of putative PRL receptors in rat brain regions as hypothalamus and choroid plexus.

The estimated M_r values for the PRL-binding units in hypothalamus and choroid plexus are somewhat lower than those found for the hormone in peripheral tissues of the rat. For instance, with the same experimental technique M_r values of 42,000–44,000 have been reported for the rat liver receptor [22]. Recent studies have shown that two forms of PRL-receptor mRNA are detectable in the rat hypothalamus [23]. The predicted M_r-values for the receptor proteins from these mRNA sequences were previously reported to be 33,000 [24] and 67,000 [25] when studied in the rat liver. However, these values are calculated from predicted amino acid sequences which do not include glycosylation. The observed size heterogeneity arose from alternative splicing of the same primary receptor mRNA transcript [26].

The salient new finding reported here is the significant decrease in PRL-binding observed in the acute phase of morphine administration, i.e. 24 h after pump implantation. At this time the maximal analgesic effect of morphine was observed prior to development of tolerance. The decrease in PRL-binding sites in the acute phase could reflect a down-regulation caused by a morphine-induced increase of the hormone secretion. The drug may act on the dopamine system in hypothalamus and thereby interfere with the mechanism controlling the PRL release [27]. However, the morphine-induced increase in PRL secretion was seen only during the acute phase of drug administration [8,9]. Studies have shown that continuous infusion of PRL in the rat from implanted miniosmotic pumps does not affect the receptor content in peripheral tissues, such as the liver [28].

On the other hand, it can not be excluded that morphine has a direct effect on the PRL receptors in certain brain areas as hypothalamus and choroid plexus. Morphine may affect the expression of the receptor at the transcriptional level. Chronic administration of opioid agonists have previously been shown to produce alterations in the gene transcript for several prepropeptides and also for peptide receptors. For instance, morphine was found to down-regulate the expression of proopiomelanocortin in the rat [29]. A similar observation was made for the prodynorphin gene in the rat brain [30]. A down-regulation of the adenosine receptor activity following morphine treatment was also reported [31]. The molecular mechanism behind these alterations are, however, still unclear and needs further elucidation.

The down-regulation of PRL receptors in the hypothalamus, as indicated by this study, is of importance for the control mechanism of the PRL secretion. A decrease in transduction of the PRL signal in this area will certainly affect the activity in the DA neurons [12], leading to an increase in the secretion of the hormone from the anterior pituitary. It is therefore tempting to speculate whether the previously described enhancement in PRL secretion following administration of opioid agonists [1–9] may result from a direct effect on PRL receptors at the hypothalamic level. This would be in agreement with the negative correlation between the plasma level of the hormone and the density of its receptors in hypothalamus as observed in this study.

The reduction of PRL-binding sites in the choroid plexus may have consequences for the passage of the hormone over the blood–brain barrier according to a previously described hypothesis. The PRL binding-sites in this region were suggested to mediate the transport of PRL from the vascular compartment into the CNS [32,33]. Thus, a down-regulation of PRL receptors in choroid plexus in the acute phase may reduce the CNS

Table 2
Association constants (K_a) and binding capacities for [^{125}I]-oPRL to male rat membranes from choroid plexus and hypothalamus collected from animals in the acute phase of morphine treatment (24h following pump implantation)

Tissue	Binding capacity (nmol/mg protein)	K_a (nmol^{-1})	M_r for oPRL--binding unit
Choroid plexus			
Control	1.96 ± 0.10	2.2 ± 0.9	36 000 ± 1100
Morphine	0.62 ± 0.02**	2.6 ± 0.5	
Hypothalamus			
Control	0.71 ± 0.05	4.5 ± 1.2	37 000 ± 1000
Morphine	0.45 ± 0.06*	5.1 ± 1.8	

Values are given as means ± S.D. (n = 3).
* $P < 0.05$, ** $P < 0.01$.

levels of the hormone at this stage of chronic morphine administration.

In conclusion, this work shows that prolactin-binding in hypothalamus and choroid plexus is significantly reduced in the acute phase of morphine dependence. At this stage the density of the PRL-binding sites in these brain regions correlated negatively to the plasma levles of the hormone. The significance of these changes in relation to the effects of morphine at this stage of its administration is not yet clear. Alterations in the PRL-binding seen during withdrawal and abstinence (Table 1) suggest an involvment of PRL receptors in the expression of both these states during the development of morphine dependence

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