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Anticholinesterase activity of prolactin: correlation with analgesia

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Abstract—The possible inhibitory effect of prolactin on serum cholinesterase activity has been examined. Prolactin given to mice inhibited the enzyme's activity in a dose-related fashion. This inhibition was not reversed by naloxone. A significant correlation was observed between the anticholinesterase and analgesic activity of prolactin. The findings suggest that prolactin may exert its cholinomimetic activity by inhibition of cholinesterase. A significant contribution by the cholinergic activity, which was independent of the opioid system, was indicated in the analgesic effect.

Cholinomimetic agents have been shown to produce analgesia and to enhance that of morphine (Karczmar 1976; Koehm & Karczmar 1978). Prolactin elicits an opioid-mediated analgesic effect (Ramaswamy et al 1983) and like morphine, the analgesic effect is influenced by cholinergic agents (Ramaswamy et al 1986). Prolactin has an inherent cholinomimetic activity, as shown by its contractile effect on guinea-pig isolated ileum (Pillai et al 1981) and enhanced intestinal transit in mice (Gopalakrishnan et al 1981), which was thought to be of a muscarinic type. However, the exact mechanism of the cholinergic action has not been resolved. One possible mechanism, i.e. inhibition of cholinesterase (ChE) enzyme activity by prolactin has been investigated by us and correlated with its analgesic action.

Method

Swiss male albino mice (25–30 g) were used.

Analgesia was assessed by any reduction in writhing movements following injection of acetic acid (0·6%, 10 mL kg^{-1} i.p.). The writhings were counted for 10 min. A significant reduction in the number of writhings represented the analgesic effect.

Estimation of serum cholinesterase activity was according to the method of Rappaport et al (1959). Animals were decapitated for collection of blood, and the serum, free from haemolysis products, was used for the assay. An indicator, *m*-nitrophenol, was used to measure the acetic acid produced by the enzymatic hydrolysis of acetylcholine. The decrease in the colour of *m*-nitrophenol was measured spectrophotometrically at 420 nm. The results were expressed as Rappaport units mL^{-1} = the amount of enzyme that liberate 1 μmol of acetic acid from acetylcholine in 30 min at 25 °C and pH 7·8.

The analgesic effect and ChE activity was assayed 30 min after the animals had been given (i.p.) 20, 40 or 80 μg kg^{-1} prolactin. To study the role of the opioid system in prolactin-induced changes in the ChE activity, the effect of naloxone 5 mg kg^{-1} (i.p.) alone and as pretreatment (10 min before prolactin 40 μg kg^{-1}) were estimated. Saline-treated animals served as control.

Drugs and chemicals used were: prolactin (Ovine, NIH, Bethesda), naloxone hydrochloride (Endo), Sigma kit No. 420 for assay of ChE activity and acetic acid (AR).

Statistical analysis was with Student's *t*-test.

Results

Prolactin, in the doses employed, produced a significant and dose-related inhibition of the writhing response (Fig. 1). The serum ChE activity was also significantly inhibited in prolactin-treated animals compared with controls. This inhibition was dose-related (Fig. 1). A significant correlation was observed (correlation coefficient (r) = 0·89) between the inhibition of writhing and ChE activity. Naloxone alone did not alter the ChE activity and pretreatment with it failed to change the inhibitory effect of prolactin on serum ChE (Table 1).

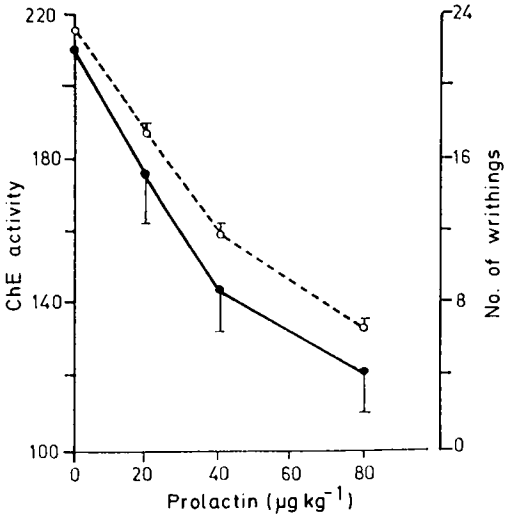

FIG. 1. Dose-related inhibition of writhing and anticholinesterase activity by prolactin. Values represent mean ± s.e.m. of six experiments. Key: ○, writhing; ●, ChE activity (Rappaport units mL^{-1}).

Table 1. Effect of naloxone alone and with prolactin on the serum cholinesterase activity in mice

Treatment mg kg^{-1} i.p.	Serum ChE activity Rappaport units mL^{-1}
Saline	216 ± 20
Prolactin 0·04	144 ± 13*
Naloxone 5	216 ± 14
Naloxone 5 + prolactin 0·04	158 ± 16*

Values represent mean ± s.e.m. (n = 6).
*$P < 0·05$ as compared with saline value.
Naloxone was administered 10 min before prolactin.

Discussion

An indirect in-vivo cholinomimetic role for prolactin is suggested since there was an increased gut motility and gastric emptying in lactating mice. A pA_2 value against atropine for prolactin was similar to that for acetylcholine thereby indicating an acetylcholine type of action for prolactin (Pillai et al 1981). Those authors have also proposed that prolactin may act either

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through muscarinic receptors or acetylcholine release. Our results show that one of the mechanism of cholinomimetic activity of prolactin is by inhibition of ChE. This anti-ChE action may be responsible for the earlier reported cholinomimetic activity of prolactin.

It is well known that anti-ChE agents like di-isopropyl fluorophosphate and physostigmine are significant analgesics in various experimental procedures (Koehm & Karczmar 1978). It is possible that the anti-ChE activity of prolactin we have found might play a part in the analgesic action. This view is supported by our observation that a significant correlation exists between anti-ChE and analgesic activity. However, although earlier studies on prolactin analgesia showed an involvement of the opioid system (Ramaswamy et al 1983) the absence of any effect of naloxone on ChE activity and its failure to reverse the anti-ChE activity of prolactin in the present work suggest that opioid mechanisms may not be involved in that action of prolactin.

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Dopamine and apomorphine do not modulate the uptake of [^{3}H]D-aspartate in the rat striatum in-vitro

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Abstract—Sodium-dependent [^{3}H]D-aspartate uptake was measured in rat striatal homogenates. The uptake was inhibited by both L- and D-glutamate, with IC50 values of 5·6 and 224 μM, respectively. Dopamine (10^{-7}–10^{-4} M), apomorphine (10^{-7} M), sulpiride (10^{-6} M) or a combination of dopamine and sulpiride were found not to affect the observed uptake of [^{3}H]D-aspartate. Thus, the in-vitro dopaminergic modulation of high affinity glutamate uptake reported in the literature is not found when [^{3}H]D-aspartate is used instead of [^{3}H]L-glutamate.

There are many studies in the literature demonstrating that the release of a neurotransmitter from its nerve endings can be modulated by other neurotransmitter systems (for review, see Chesselet 1984). In the case of glutamatergic neurons, it has been demonstrated that potassium-stimulated release of tritium from striatal slices preincubated with [^{3}H]L-glutamate is inhibited by dopamine and its agonists in a manner that can be blocked by dopamine receptor antagonists (Mitchell & Doggett 1980; Rowlands & Roberts 1980). A dopaminergic modulation of the high affinity uptake of [^{3}H]L-glutamate into rat striatal (but not frontal cortical) homogenates has also been reported, with 10^{-7} M dopamine, apomorphine and bromocriptine producing 34, 19 and 20% inhibition, respectively, of the uptake (Nieoullon et al 1982, 1983).

One of the disadvantages associated with the use of [^{3}H]L-glutamate in these types of studies is the rapid metabolism of the amino acid, a problem that can be avoided by the use of [^{3}H]D-aspartate, which uses the same neuronal uptake system as glutamate (see Logan & Snyder 1972; Taxt & Storm-Mathisen 1984). In the present study, the effects of dopamine, apomorphine and sulpiride on the uptake of [^{3}H]D-aspartate into rat striatal homogenates has been investigated.

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Materials and methods

St. Mary's bred male and female Fisher rats, 200–300 g, were used in the study. The rats were decapitated and their brains rapidly removed, placed on ice, and the striata dissected. The uptake experiments were undertaken by a method based on that of Storm-Mathisen (1977) and similar to that used by Nieoullon et al (1982, 1983), with the exceptions that the whole striatum (rather than the rostral third) was used, [^{3}H]D-aspartate was used in place of [^{3}H]L-glutamate, the incubations were at 37 °C rather than at 25 °C, and the reactions were terminated by filtration rather than centrifugation. Briefly, the striata were homogenized 1:20 (w/v) in 0·32 M ice-cold sucrose, and 50 μL of homogenate added to incubation wells containing the dopamine agonist/antagonist under test dissolved in 460 μL Krebs phosphate buffer, pH 7·4. Reactions were started by the addition of 50 μL [^{3}H]D-aspartate (final concentration 65·7 nM at a specific activity of 14 Ci mmol^{-1}). After a 3 min incubation at 37 °C, the reactions were terminated by rapid filtration through Whatman GF/B filter papers, using a Brandel Cell Harvester. The filters were washed four times with isotonic saline and then allowed to dry before determination of the tritium content by liquid scintillation spectroscopy. Sodium-free uptake of [^{3}H]D-aspartate was determined by replacement of sodium salts in the Krebs buffer by iso-osmotic Tris HCl, pH 7·4.

[^{3}H]D-Aspartate was obtained from Amersham International plc, Amersham, UK. Dopamine hydrochloride, apomorphine