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Modifications in prolactin binding capacity in the rat liver induced by non-steroidal anti-inflammatory drugs

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Modifications in prolactin specific binding in the rat liver induced by different non-steroidal anti-inflammatory drugs (indomethacin, piroxicam, ketoprofen, phenylbutazone, mefenamic acid and acetylsalicylic acid) have been studied. All caused a dose-dependent inhibition of prolactin binding capacity whereas no change was found in dissociation constant values. The inhibitory effect is reversible and highly specific, since insulin binding to the same membrane preparation is not affected. The degree of inhibitory activity on prolactin binding is related to the anti-inflammatory activity of each drug, which supports the hypothesis of an involvement of prostaglandin synthesis.

An interaction between a hormone molecule and a specific binding site on the surface membrane of target cells is recognized to be the initial step in the mechanism of action of polypeptide hormones. Specific binding sites for prolactin have been described in a variety of tissues, including the liver, mammary gland, adrenal, kidney, ovary and brain of many species (Posner et al 1974; Shiu & Friesen 1974; Poindexter et al 1979; Calvo et al 1981; Mountjoy et al 1980; Di Carlo & Muccioli 1981). The binding activity of these sites was shown to be sensitive to trypsin and phospholipase C digestion, suggesting that protein and phospholipid moieties are essential for the interaction with prolactin (Shiu & Friesen 1974).

Recently, it has been observed that indomethacin is able to reduce the number of prolactin receptors detectable in the mouse liver membranes (Knazek et al 1981). This activity was correlated with a decrease in liver membrane fluidity induced by indomethacin through its interference on prostaglandin synthesis.

In our researches we have studied in detail the modifications in prolactin binding capacity in the rat liver induced by different non-steroidal anti-inflammatory drugs, demonstrating in particular the specificity and reversibility of such an effect.

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MATERIALS AND METHODS

Animals

Female albino Sprague-Dawley rats, 160–180 g, were housed at constant temperature (20 °C) under fixed 12 h light and dark cycles with free access to food and water.

Effect of repeated treatments with indomethacin

Thirty-six rats in groups of 6 were treated thus: group 1 was given one dose of indomethacin (3 mg kg^{-1} i.m.), groups 2, 3, 4 and 5 received the same dose repeated respectively two, three, four and five times, at 4 h intervals. Group 6 had no treatment and served as control. The animals were decapitated 8 h after the last treatment. Livers were removed for membrane preparation.

Time-course of indomethacin effect

To assess the time-course of indomethacin effect on prolactin specific binding, four groups of 6 rats were given indomethacin (1·5 mg kg^{-1} i.m.) repeated five times at 4 h intervals, then decapitated at 8, 16, 36, 60 h after the last injection. At 8 and 60 h six untreated control rats were killed.

Effect and dose-response ratio of different non-steroidal anti-inflammatory drugs

The animals were divided into a control and twenty-one test groups (5 rats per group) which received either indomethacin (1·5, 3 or 6 mg kg^{-1} i.m.),

ketoprofen (3, 5 or 8 mg kg^{-1} i.m.), piroxicam (3, 6 or 9 mg kg^{-1} i.m.), phenylbutazone (35, 50 or 70 mg kg^{-1} i.m.), mefenamic acid (30, 60 or 90 mg kg^{-1} oral), acetylsalicylic acid (50, 70 or 100 mg kg^{-1} i.m.) or paracetamol (32·5, 65 or 100 mg kg^{-1} oral) respectively. Each dose was repeated 5 times at 4 h intervals. All rats were killed 8 h after the last treatment.

Membrane preparation

Livers were homogenized in 0·3 M sucrose (10 vol) at 4 °C using a Polytron PT-10. The homogenates were centrifuged at 15 000*g* for 20 min at 4 °C and the resulting supernatant decanted. This was centrifuged at 105 000*g* for 60 min at 4 °C to prepare a microsomal pellet according to Posner et al (1974). The resulting pellet was suspended in ice-cold 25 mM Tris-HCl 10 mM $MgCl_2$ (pH 7·4) using a hand-operated Potter-Elvejhem type homogenizer and stored at −20 °C until assayed for protein determination (Lowry et al 1951) and for hormone binding studies.

Hormones and iodination

Ovine prolactin (NIAMDD-oPRL-I-1 35 i.u. mg^{-1}) was kindly provided by the Pituitary Hormone Distribution Program of the NIADDK. Porcine insulin (25 i.u. mg^{-1}) was purchased from Schwarz/Mann.

Ovine prolactin was iodinated (^{125}I-labelled ovine prolactin S.A. 42–53 mCi mg^{-1}) using the method of Bolton & Hunter (1973) in which radioactive iodine is introduced by reaction of free amino groups of the protein with *N*-succinimidyl 3(4-hydroxy-,5-[^{125}I]-iodophenyl)propionate (Amersham International).

Porcine insulin, iodinated (^{125}I-labelled insulin S.A. 87 mCi mg^{-1}) by the chloramine-T method (Hunter & Greenwood 1962), was purchased from New England Nuclear (Boston, MA, USA).

Hormone binding studies

The prolactin binding studies were made as described by Shiu et al (1973) with slight modifications, with the microsomal pellet, which is referred to as the main membrane preparation, containing the prolactin receptors. In the binding studies, about 80 000 counts min^{-1} of ^{125}I-labelled ovine prolactin (0·9–1·1 ng) was added to each tube, containing 0·2 mg membrane protein in a final volume of 0·5 ml assay buffer (25 mM Tris-HCl, 10 mM $MgCl_2$, 0·1% bovine serum albumin, pH 7·4). After 16 h of incubation at 20 °C, the binding reaction was stopped by addition of 3 ml of the ice-cold assay buffer. Bound and free ^{125}I-labelled ovine prolactin were separated by low-speed centrifugation (1500*g*) for 30 min at 4 °C. The supernatant was decanted and the membrane pellet was counted for radioactivity in a Packard auto-gamma counter. The specific binding was evaluated as the difference between binding in the absence and the presence of excess unlabelled ovine prolactin (2 μg ml^{-1}) expressed as percent of the total counts added to incubation medium.

Scatchard analysis (Scatchard 1949) of the prolactin binding to liver membranes was also performed by transformation of binding data from the competition studies with increasing concentrations of unlabelled ovine prolactin mixed with a fixed amount of tracer. The dissociation constant (Kd) and binding capacity (n) were determined for prolactin concentrations between 1·6 and 100 ng ml^{-1}.

Specificity of indomethacin induced modifications on the prolactin binding

To determine the specificity of indomethacin effect, liver membranes from rats treated with indomethacin were also assayed for the binding with insulin, a hormone possessing specific receptors located on the plasma membrane (Posner et al 1978).

The specific binding of ^{125}I-labelled insulin was estimated as for ^{125}I-labelled ovine prolactin, the incubation being at 4 °C for 48 h.

Non-linear Scatchard plots of insulin binding to liver membranes were resolved by the limiting slopes technique of Hunston (1975), assuming two independent classes of non-cooperative binding sites.

Histological examination. Samples of liver for light microscopy were fixed in Bouin's fixative and embedded in paraffin wax. Samples sections (5 μm thick) were stained with haematoxylin and eosin.

Effects of indomethacin and ketoprofen in-vitro on the prolactin binding. Specific binding of ^{125}I-labelled ovine prolactin to liver membranes from untreated rats was determined in the presence of varying concentrations (0·1, 1, 10 and 100 μg ml^{-1}) of indomethacin or ketoprofen, following the method described.

Statistical analysis. All results are expressed as group arithmetic means and standard deviation of the means. Statistical comparison between group means were carried out using the Student's *t*-test. The ID50 value of the different drugs on prolactin binding was determined by probit analysis.

Drugs. Indomethacin (Sigma Chemicals, U.S.A.) and piroxicam (Pfizer Italiana, Roma) were dissolved in phosphate buffer (pH 8·0). Ketoprofen (Farmitalia, Milan), phenylbutazone (Sigma Chemicals, U.S.A.) and acetylsalicylic acid as lysine acetylsalicylate (Maggioni, Milan) were dissolved in distilled water. Mefenamic acid (Schiapparelli, Torino) and paracetamol (Sigma Chemicals, U.S.A.) were suspended in 0·5% carboxymethylcellulose. Controls were treated with an equivalent volume of vehicle that was used to administer the drugs.

RESULTS

Effect of repeated treatments with indomethacin. A significant inhibition of prolactin specific binding appears after three repeated treatments with indomethacin (Fig. 1). This effect further increases after 4 and 5 treatments.

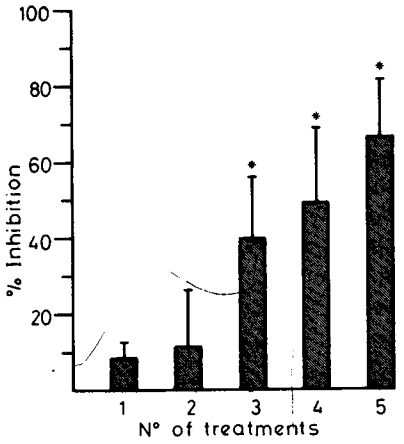

FIG. 1. Effect of repeated treatments with indomethacin (3 mg kg^{-1} i.m. every 4 h) on prolactin specific binding to liver membranes of rats killed 8 h after the last treatment. (*) $P < 0.01$ vs controls.

Time-course of indomethacin effect. The inhibiting effect of indomethacin on prolactin binding to liver membranes was reversible; 60 h after the last treatment the binding capacity of liver membranes was completely restored (Fig. 2).

Effect and dose-response ratio of different non-steroidal anti-inflammatory drugs. All the non-steroidal anti-inflammatory drugs induced a dose-related inhibition of prolactin binding to rat liver membranes. The inhibiting dose 50 (ID50) values are in Table 1. The highest activity was shown by

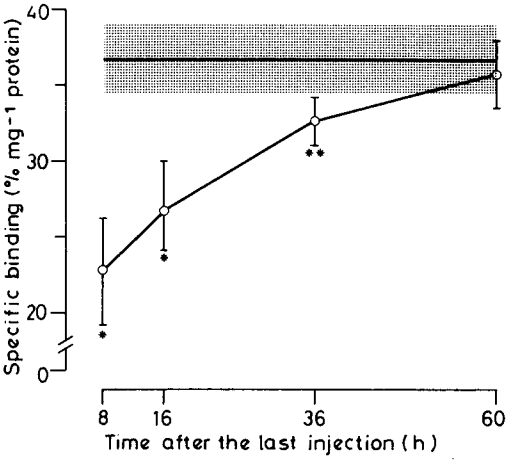

FIG. 2. Time-course of indomethacin effect on prolactin specific binding to rat liver membranes. The animals were treated with 1·5 mg kg^{-1} i.m. of indomethacin repeated five times at 4 h intervals and killed at various times after the last injection. Shaded area: range of basal values (untreated animals). (*) $P < 0.001$; (**) $P < 0.05$ vs controls.

indomethacin, the lowest by acetylsalicylic acid (paracetamol was inactive).

The inhibiting effect on the binding of the drugs (Table 1) is related to their anti-inflammatory activity evaluated on rat paw oedema and their anti-prostaglandin synthetase activity.

Specificity of indomethacin induced modifications on the prolactin binding. Scatchard analysis of the binding of ^{125}I-labelled ovine prolactin to liver membranes show no evident difference in dissociation constant observed in liver from untreated or

Table 1. Inhibitory activities (ID50) of different non-steroidal anti-inflammatory drugs on prolactin binding (PRL-B) to rat liver membranes compared with their ability in inhibiting the rat paw oedema (RPO) and their anti-prostaglandin synthetase activity (PGS).

Drugs	PRL-B ID50 value mg kg^{-1}	RPO ID50 comparative value (indomethacin = 1)	PGS ID50 value[d] μM
Indomethacin	2·5	1[a]	0·6
Ketoprofen	5·4	3·7[b]	0·9
Piroxicam	5·9	2·5[c]	0·8
Phenylbutazone	47	15·4[a]	12·0
Mefenamic acid	55	9·7[a]	—
Acetylsalicylic acid	90	23·1[a]	110
Paracetamol	>100	Inactive[a]	—

[a] Quoted by Flower et al (1972).
[b] Quoted by Lombardino et al (1975).
[c] Quoted by Wiseman et al (1976).
[d] Quoted by Carty et al (1980).

indomethacin-treated rats, whereas the number of binding sites varied, showing an evident dose-related decrease in treated rats.

Conversely, dissociation constants and binding capacities of ^{125}I-labelled insulin to the same liver membranes were not affected by these treatments.

Histological examination. No evident histological modifications in comparison with control rats were found in the liver of treated animals 8 h after the 5th administration.

Effects of indomethacin and ketoprofen in-vitro *on the prolactin binding*. Indomethacin or ketoprofen at all concentrations were unable to modify the specific binding of ^{125}I-labelled ovine prolactin to liver membranes.

DISCUSSION

Membrane-bound receptors for prolactin were found in rat liver, but their physiological role has yet to be resolved. It has been reported that prolactin has significant effects on rat liver. The stimulation of RNA synthesis (Chen et al 1972), the increase in activity of ornithine decarboxylase and thymidine kinase in the liver of prolactin-treated animals (Richards 1975; Thomson & Richards 1978), the production of somatomedine in response to prolactin (Francis & Hill 1975) and the regulation of the oestradiol receptor in the liver (Chamness et al 1975), suggest that prolactin binding by liver is a biologically important event. Furthermore, the number of prolactin receptors in the rat liver has been shown to be sexually differentiated; it is much greater in females than in males and increases further during pregnancy (Kelly et al 1974).

Our results show that the treatment with different non-steroidal anti-inflammatory agents induces a dose-dependent decrease of prolactin binding capacity in rat liver membranes. Such changes in binding capacity are reversible and highly specific for prolactin receptors. Evidence for specificity was provided by failure of anti-inflammatory drugs to alter insulin binding to the same membrane preparation. The histological examination of the liver was unable to detect any evident modification. These results seem to preclude the hypothesis of a general non-specific effect on membrane proteins resulting in a reduced binding of polypeptide hormones.

The degree of inhibitory activity on prolactin binding to rat liver membranes displayed by the different drugs used was always in good agreement with the anti-inflammatory activity and with the inhibitory activity on prostaglandin biosynthesis showed by each agent. Paracetamol, which has analgesic and antipyretic effects, but not anti-inflammatory activity (Flower & Vane 1974), was inactive on prolactin binding.

Furthermore, the lack of activity of indomethacin and ketoprofen, added in-vitro to liver membranes during incubation, does not support a direct effect of the two drugs on the membrane-bound receptors. Since these drugs are potent inhibitors of prostaglandin synthesis in-vitro these results suggest that the reduction in binding seen after in-vivo treatment could be the result of changes secondary to this inhibition.

Our finding seems to be in agreement with the results obtained by Knazek et al (1981) in the mouse liver where an inhibition of prostaglandin synthesis simultaneously caused a rapid suppression of prolactin receptors and an increase in membrane microviscosity. Changes in membrane fluidity could induce positional or conformational modifications of some membrane components like protein receptors, altering their functionality and detectability (Dave et al 1981a, b). Although the modifications on prolactin receptors in the liver have been obtained with doses of anti-inflammatory drugs much higher than those used in therapy, we used only acute treatments (five doses over 24 h), and it is possible that for chronic treatment therapeutic doses could interfere with prolactin activity through changes in the hormone receptors.

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