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18. Steroids in the Neuroendocrine System

MODULATION OF BRAIN PROGESTIN AND GLUCOCORTICOID RECEPTORS BY UNSATURATED FATTY ACID AND PHOSPHOLIPID

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Summary—In an attempt to learn how nonsteroidal factors modulate brain progestin and glucocorticoid receptors, the effects of saturated and unsaturated fatty acids, and phosphatidylinositol on the binding of [^{3}H]R5020 or [^{3}H]dexamethasone, determined by sucrose density gradient and gel filtration on LH20, were examined in the cerebral cortical cytosol from 10-day-old female rats which contain a considerable amount of progestin and glucocorticoid receptors. Unsaturated fatty acids such as oleic (C18:1), arachidonic (C20 : 4) and docosahexaenoic acid (C22 : 4) depressed the [^{3}H]R5020 or [^{3}H]dexamethasone binding in increasing order, but saturated fatty acids had no effect. Arachidonic and docosahexaenoic acids, which were strong inhibitors, lowered the binding dose dependently. The fatty acid inhibition on brain progestin and glucocorticoid receptors was thus a function of acid dose and degree of acid unsaturation. Interestingly, prostaglandin D_2 did not show any effect. Among phospholipids tested the inhibitory effect of phosphatidylinositol on the [^{3}H]R5020 binding was evident, but no significant effect was found with phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine or sphingomyelin. The phosphatidylinositol inhibition was dose dependent. Analysis on kinetics and Scatchard plot have revealed the noncompetitive type of inhibition by arachidonic acid and phosphatidylinositol. From these results it is suggested that the unsaturated nonestrified fatty acid, arachidonic acid, and phosphoinositides modulate the brain progestin and, possibly, glucocorticoid receptors through their binding at sites different from steroid binding sites on the respective receptor molecules.

INTRODUCTION

Steroidal and non-steroidal factors regulate and/or modulate steroid hormone receptors in the brain and hypophysis [1–4]. Unsaturated nonestrified fatty acids have been reported to modulate positively or negatively estrogen binding in the immature rat uterus [5, 6] and human uterus, breast cancer, and melanoma tissue [7], implying some role of the fatty acid as a possible modulator of the action of estrogen on the target tissue.

Brain is known as the richest source of phospholipid, and unsaturated fatty acids, which are mainly contained in membrane phospholipid and released on signal stimulation [8–13]. It is thus interesting to learn how unsaturated fatty acids and phospholipids affect steroid hormone receptors in the brain. We preliminarily reported that the binding of [^{3}H]R5020 to progestin receptor in the neonatal cortex was inhibited by *in vitro* addition of unsaturated fatty acids. The inhibition was a function of acid dose and acid saturation degree. Arachidonic acid strongly competed. Arachidonic acid is implicated in the regulatory and/or modulatory mechanisms of female sex steroid hormone receptors in the brain [14, 15].

In this study, we report further results on the modulatory effects of unsaturated fatty acids and phospholipids on specific progestin binding in the cytosols of the neonatal rat cerebral cortex, which contains a considerable amount of estrogen uninducible progestin receptors [2, 16]. It is also reported that the fatty acid inhibition on the cortical glucocorticoid receptor is similar to that on the progestin receptor.

MATERIALS AND METHODS

Animals

Female rats of the Wistar strain (Animal Breeding Laboratories, Ohmiya, Saitama, Japan) were used at 10 days of age, because progestin receptors reach a maximum at this age of life [16].

Preparation of brain cytosols

All animals were decapitated, and the cerebral cortex was dissected from the parietal and frontal portion, as previously described [17]. All tissues were washed in ice-cold 10% glycerol–10 mM Tris buffer (pH 7.4) containing 1 mM sodium EDTA and 12 mM thioglycerol (10% glycerol–TET buffer), blotted on filter paper, and weighed.

The cortical tissues were homogenized in 2 vol (wt/vol) 10% glycerol–TET buffer with a Teflon pestle in a homogenizer (Takashima, Tokyo, Japan) (five strokes, 600–1000 rpm, 3 min) at 4°C. The homogenates were then centrifuged at 105,000 **g** for 1 h in a Beckman L5-50 centrifuge, and the supernatant (cytosol) was obtained.

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Sucrose gradient centrifugation

The supernatant fraction, cytosol (0.31–0.65, 5–6.7 mg protein), was mixed with several concentrations of long-chain fatty acids or phospholipids in 5–10 μl ethanol, and incubated with gentle shaking at 0–4°C for 15 min. Then [^{3}H]R5020 or [^{3}H]dexamethasone was added and incubated with gentle shaking at 0–4°C for 4 h. The volume of ethanol did not exceed 5% of cytosol incubated. In order to absorb unbound radioactivity, a 1.0-ml suspension of dextran-coated charcoal (0.5% Norit A, 0.005% Dextran T70 in 10% glycerol–Tris buffer pH 8.0, at 4°C) was centrifuged for 10 min at 1500 ***g*** and supernatant was decanted off and the pellet at the bottom was dried. Then the incubation mixture was added on the ice-cold dextran-coated charcoal pellet and vortexed, and incubated for 15 min at 4°C (the pre-DCC absorption). After centrifugation for 10 min at 1500 ***g*** in a refrigerated centrifuge, an aliquot of the supernatant was layered on a sucrose density gradient (4.0–4.8 ml, 5–20% linear) in 10% glycerol–Tris buffer, pH 7.4, and centrifuged for 18 h at 45,000 rpm in a Beckman Model L5-50 ultracentrifuge with a SW 50.1 rotor, at 4°C. The above procedures were performed in a cold room at 4°C. The pH of the buffer was determined at room temperature.

After gradient centrifugation, the gradients were fractionated into 0.2-ml fractions with an ISCO Model 640 Density Gradient Fractionator (Instrumentation Specialities Co., Lincoln, NY). To determine apparent sedimentation coefficients (S) by the method of Martin and Ames [18], crystalline BSA (4.6S), yeast alcohol dehydrogenase (7.6S), and beef liver catalase (11.3S) were used as standards. Aliquots (0.2 ml of the fractionate) were transferred into a scintillation vial and counted.

In some cases, for absorption of unbound radioactivity, the DCC treatment was done in each fraction after gradient centrifugation (the post DCC absorption). Briefly, the incubation mixture (0.3–0.5 ml) was layered on a sucrose density gradient (4.5–4.8 ml, 5–20% linear) in 10% glycerol–TET buffer, and centrifuged for 18 h at 45,000–50,000 rpm in a Beckman model L3-50 or L5-50 ultracentrifuge with a SW50.1 rotor, at 4°C. A 1.0-ml suspension of dextran-coated charcoal (DCC, 0.25% Norit A, 0.0025% dextran T70 in 10% glycerol–TET buffer, pH 7.4, at 4°C) was added to each fraction (0.2-ml aliquots), mixed, and incubated for 15 min at 4°C followed by centrifugation for 10 min at 1500 ***g*** in refrigerated centrifuge. An aliquot of the supernatant was transferred into scintillation vial and counted.

Measurement of progestin or dexamethasone binding sites by gel filtration on LH-20

Three-hundred-μl aliquots of the cytosol were incubated with [^{3}H]R5020 (4.1–4.8 nM) in the presence or absence of different concentrations of fatty acids or phospholipids, at 0–2°C, overnight. Macromolecular bound ^{3}H-steroid was separated from free ^{3}H-steroid by gel filtration on LH20 [16, 19]. Briefly, 250-μl samples were dispensed onto columns that had been equilibrated with 0.5 TEGTK. The samples were washed into the columns with 150 μl 0.5 TEGTK. After sample application, the protein peak was eluted into scintillation vials with 800 μl 0.5 TEGTK. A 10-μl sample of the incubated was taken for estimation of the total [^{3}H]R5020 concentration. Ten ml of toluene scintilation fluid were added and radioactivity was counted. Specific binding was calculated by subtracting nonspecific binding ([^{3}H]R5020 plus a 100-fold excess of R5020) from total binding. Results are expressed as ^{3}H-steroid specifically bound (fmoles) per mg protein. The limits of detection of the assay method were 3 fmol/mg protein for brain.

For Scatchard analysis of specific [^{3}H]R5020 binding to the neonatal cortical receptor [20], the cytosols (0.31 ml, 300 μg protein) were incubated with graded concentrations of [^{3}H]R5020 in the range 0.15–4.2 nM in the presence or absence of inhibitor. Dissociation constants (K_{ds}) were calculated from the Scatchard plot. The Lineweaver–Burk plot was also employed for analysing the nature of inhibitory binding.

The number of dexamethasone binding sites in the brain was determined by gel filtration on LH20 in a way similar to the progestin receptor assay [16, 19, 21].

Miscellaneous

Cytosol protein concentrations were measured after charcoal treatment by the method of Lowry *et al.* [22], using BSA as standard. Nuclear pellets were hydrolyzed by 0.5 M $HClO_4$, and DNA was quantitated by the method of Burton [23], using calf thymus DNA as the standard.

Radioactivity was counted in 10-ml scintillation fluid, consisting of 4 g omnifluor (New England Nuclear, Boston, MA), dissolved in 666 ml toluene and 333 ml Triton X-100, in an Aloka model LSC-900 scintillation counter (Aloka Co., Mitaka, Tokyo, Japan) or a Packard model 300C scintillation counter.

Chemicals

Redistilled and purified solvents and reagent grade chemicals were used in all experiments. [17α-Methyl-^{3}H]17α-, 21-dimethyl-19-nor-4, 9-pregnadiene-3, 20-dione([^{3}H]R5020, SA, 87.1 Ci/mmol) and [^{3}H]dexamethasone (SA, 27 Ci/mmol) were obtained from New England Nuclear Corp., and purified by TLC as previously described [24]. Saturated and unsaturated fatty acids and phospholipids were purchased from Funakoshi Chemicai Co. (Tokyo, Japan). Prostaglandin D_2 was supplied by Ono Pharmaceutical Co. (Osaka, Japan). Steroids

were supplied by Steraloids Inc. (Pawling, NY), Sigma Chemical Co. (St Louis, MO), and Ikapharm (Jerusalem, Israel). Crystalline BSA (Nutritional Biochemical Corp., Cleveland, OH), yeast alcohol dehydrogenase (Sigma Chemical Co.) beef liver and catalase, 2x crystallized (Sigma Chemical Co.) were used. Sucrose, glycerol, and thioglycerol were purchased from Merck (Darmstadt, Federal Republic of Germany), and sodium EDTA from Sigma Chemical Co. Dextran T70 (Pharmacia Fine Chemicals, Piscataway, NJ), Norit A (American Norit Co.), and LH20 (Pharmacia Fine Chemicals) were used.

RESULTS

I. *Effects of fatty acids*

Effects of various fatty acids on progestin and dexamethasone binding

Sucrose density studies. The effects of saturated and unsaturated fatty acids on [^{3}H]R5020 binding to progestin receptor in the neonatal cortical cytosols were investigated by sucrose density gradient centrifugation. The concentration of fatty acids was 0.40–0.44 mM. As shown in Fig. 1, the peak of progestin binding in the 8S region was inhibited by unsaturated fatty acids; oleic, arachidonic and docosahexaenoic acids in increasing order of increased number of carbon and unsaturation. No apparent inhibition was found with saturated stearic acid (C18:0). Arachidonic and docosahexaenoic acids strongly competed for the binding sites.

In this context, it is interesting that prostaglandin D_2 did not show any significant effect on [^{3}H]R5020 binding (figure not shown).

Gel filtration studies. The effects of various fatty acids on the binding of [^{3}H]R5020 or [^{3}H]dexamethasone in the neonatal cortical cytosols were examined by gel filtration on LH20. As shown in Fig. 2, the binding of [^{3}H]R5020 or [^{3}H]dexamethasone was inhibited by unsaturated fatty acids, but not by saturated palmitic and stearic acids. These results were consistent with those of gradient studies.

Effects of graded doses of arachidonic acids on progestin and dexamethasone binding in the neonatal cortical cytosol

Sucrose density studies. Graded concentrations of arachidonic acid were added *in vitro* in the incubation mixture (cytosol protein 5–6 mg). The [^{3}H]-R5020 binding peak was abolished with arachidonic acid at 210 μM, and inhibited considerably at 110 μM. No apparent effect was seen at 55 μM (figure not shown).

The peak of [^{3}H]dexamethasone binding was gradually depressed with increasing concentrations of arachidonic acid (Fig. 3).

Gel filtration studies. Specific progestin binding in the neonatal cortical cytosol (300 μg protein) with

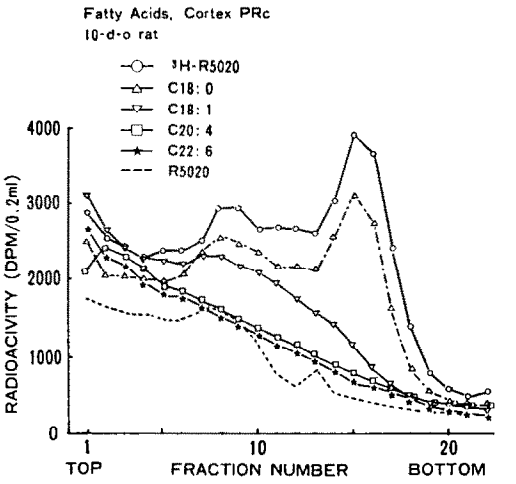

Fig. 1. Effects of unsaturated fatty acids on progestin binding to the progestin receptor in the neonatal rat cortex. Representative sucrose density gradient patterns of 10-day-old female rat cerebral cortical cytosols incubated *in vitro* with [^{3}H]R5020 in the presence of fatty acids. Cytosols (0.65 ml, 6.71 mg protein) were preincubated with or without fatty acid (0.40–0.44 mM) dissolved in 10 μg ethanol and further incubated with [^{3}H]R5020 (5 μl ethanol, 3.2 nM, SA 86 Ci/mmol) for 4 h at 0°C. To absorb unbound [^{3}H]R5020 the incubation mixture was treated with the dextran-coated charcoal pellet. The supernatant (0.53 ml, 5.35 mg protein) was then layered on 4 ml sucrose density gradients (5–20%) containing 10% glycerol, 10 mM Tris-HCl, and 1 mM sodium EDTA which were centrifuged at 45,000 rpm for 18 h in a SW50.1 rotor in a Beckman L5-50 model ultracentrifuge. BSA and alcohol dehydrogenase (ADG) were standard enzymes for the determination of sedimentation coefficients. ○, [^{3}H]R5020 alone; △, C18;0, stearic acid; ▽, C18:1, oleic acid; □, C20:4, arachidonic acid; *, C22:6, docosahexaenoic acid; ---, [^{3}H]R5020 in the presence of unlabeled R5020.

increasing concentrations of arachinate from 1.53 to 153 μM was measured by gel filtration on LH20 (Fig. 4). The binding was dose dependent at the concentration of 7.7 μM or more. At 76.5 μM or more the binding was almost abolished. In contrast, no inhibition was found by saturated fatty acid, palmitic acid (C16:0). Inhibitory potency as expressed by the concentration for 50% maximum inhibition (K_i) by arachidonate was 16.5 μM (mean, $N = 2$).

These data from density gradient and gel filtration studies indicate a dose-dependent inhibition of arachidonic acid on progestin and dexamethasone binding in the neonatal cortical cytosol.

Nature and binding kinetics of arachidonate inhibition

Scatchard analysis was performed to examine the nature and binding kinetics of arachinate inhibition of [^{3}H]R5020 binding to its cytosol receptor in the neonatal cortical cytosols (Fig. 5, left panel). Specific binding was determined by gel filtration on LH20.

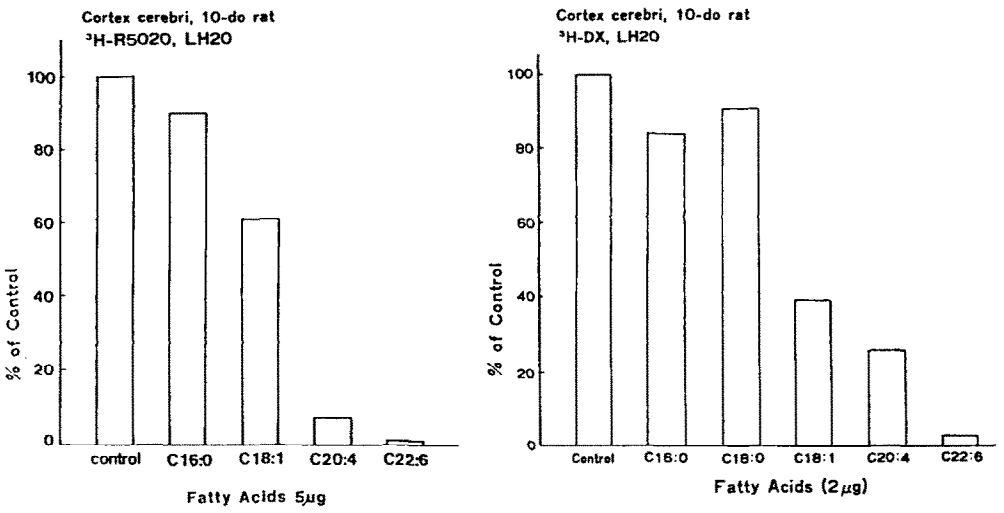

Fig. 2. The inhibition of progestin and dexamethasone binding in the neonatal cerebral cortex by unsaturated fatty acids. The cytosols (0.2 ml, 200 μg protein) were incubated with [³H]R5020 (4.8 nM) or [³H]dexamethasone (1.0 nM) for 4 h at 0°C in the presence or absence of saturated and unsaturated fatty acids (110 μM for [³H]R5020 binding; 22 μM for [³H]dexamethasone binding). Specific binding was measured by gel filtration on LH20. The detail of the experiment was described in the text. The number of binding sites (fmol/mg protein) in the absence of the fatty acid (the control) was taken as 100%. Left panel; with [³H]R5020, right panel; with [³H]dexamethasone. C16:0, Palmitic acid; C18;0, stearic acid; C18;1, oleic acid; C20:4, arachidonic acid; C22;6, docosahexaenoic acid.

While the maximum number of available binding sites was clearly decreased in the presence of arachidonate, the equilibrium dissociation constants (K_{ds}) of the progestin binding in the presence of 15.3 and 38.3 μM arachidonate were 0.26 and 0.36 nM, respectively. The values were almost the same as those for the controls without arachidonate (0.30–0.38 nM).

Analysis of the same data utilizing a double reciprocal plot (Lineweaver–Burk plot) revealed a pattern of non-competitive inhibition by arachidonate (Fig. 5, right panel).

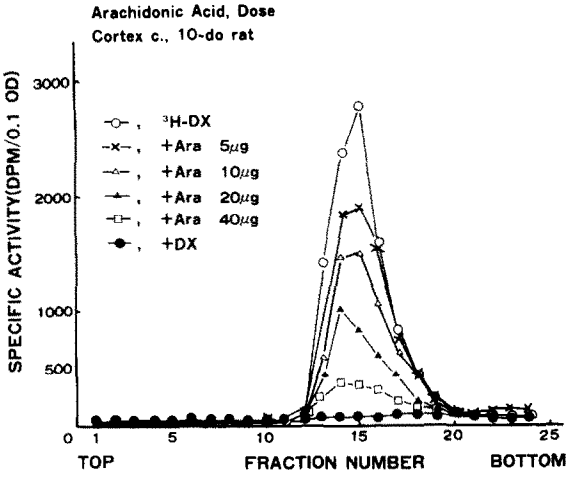

Fig. 3. Effects of grades of doses of arachidonic acid on dexamethasone binding in the neonatal rat cortex. Representative sucrose density gradient patterns of 10-day-old female rat cerebral cortical cytosols incubated *in vitro* with [³H]dexamethasone in the presence of different doses of arachidonic acid. Cytosols (0.31 ml, 5.0 mg protein) were preincubated with or without of 5, 10, 20 or 40 μg of arachidonic acid dissolved in 10 μl of ethanol and further incubated with [³H]dexamethasone (5 μl ethanol, 5.9 nM, SA 27 Ci/mmol) for 4 h at 0°C. After treatment of the incubation mixture with dextran-coated charcoal pellet, the mixture (0.23 ml, 3.83 mg protein) was layered on sucrose density gradients (5–20%) containing 10% glycerol, 10 mM Tris–HCl, and 1 mM sodium EDTA which were centrifuged at 45,000 rpm for 18 h in a SW50.1 rotor in a Beckman L5-50 model ultra-centrifuge. BSA and alcohol dehydrogenase (ADG) were standard enzymes for the determination of sedimentation coefficients. Specific radioactivity was expressed as dpm per 0.2 ml/0.1 OD 260 mu. ○, [³H]Dexamethasone (DX) alone; ×, plus 5 μg arachidonic acid (55 μM); △, plus 10 μg arachidonic acid (110 μM); △, plus 20 μg arachidonic acid (210 μM); □, plus 40 μg arachidonic acid (420 μM); ○, in the presence of unlabeled DX.

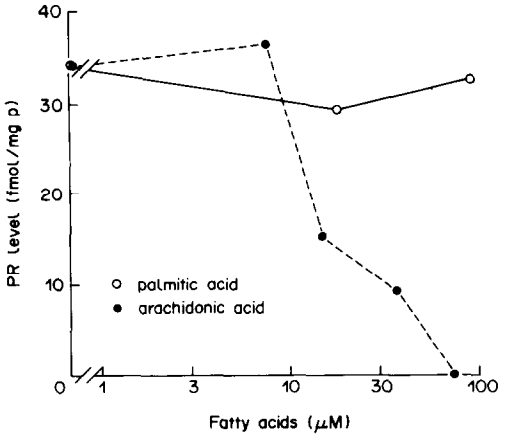

Fig. 4. Inhibition of specific binding of [^{3}H]progestin to its cytosol receptor from neonatal female rat cerebral cortex by arachidonate. The cytosol (0.2 ml, 300 μg protein) was incubated with [^{3}H]R5020 (4.8 nM) at 0°C for 4 h in the presence or absence of increasing concentrations of arachidonate (7.7–15.3 μM) and palmitic acid (18.2–91.0 μM). Specific binding to the progestin receptor was measured by gel filtration on LH20. The detail of the experiment was described in the text. Each point represents the mean of two determinations. The K_i value for arachidonate inhibition was calculated to be 14.5 μM.

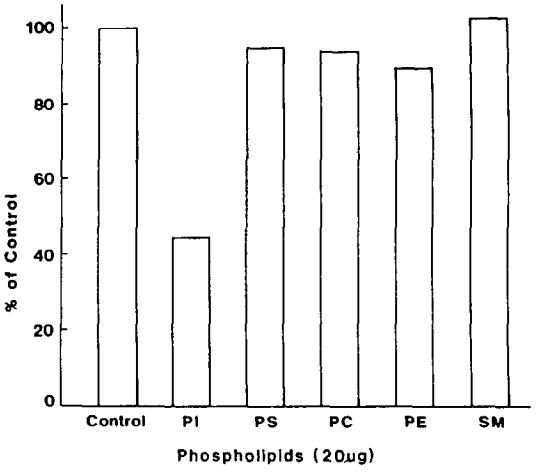

Fig. 6. The effects of phospholipid on progestin binding in the neonatal rat cerebral cortex. The cytosols (0.2 ml, 300 μg protein) were preincubated with various phospholipids (20 μg, 111 μM) and then further incubated with [^{3}H]R5020 (4.8 nM). Specific [^{3}H]R5020 binding was measured by the gel filtration (LH20) method. The experimental procedures were described in the text. The number of binding sites (fmol/mg protein) in the absence of phospholipid (the control) was taken as 100%. PE, Phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI. Phosphatidylinositol; SM, syphingomyelin.

II. *Effects of phospholipids*

Effects of phospholipids on progestin binding

Various phospholipids were added into the incubation mixture, and specific [^{3}H]R5020 binding was determined by gel filtration on LH20.

As clearly shown in Fig. 6, phosphatidylinositol (PI) inhibited the [^{3}H]R5020 binding, but no significant effect was found with phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine or sphingomyelin.

Effects of phosphatidylinositol on progestin receptor

Phosphatidylinositol (PI), a strong inhibitor of brain progestin receptor, is particularly interesting in relation to the mechanism of action of hormone [29]. We have investigated the effect of PI on progestin binding in the brain.

Graded doses of PI on specific [^{3}H]R5020 binding in the neonatal cortical cytosols were first examined.

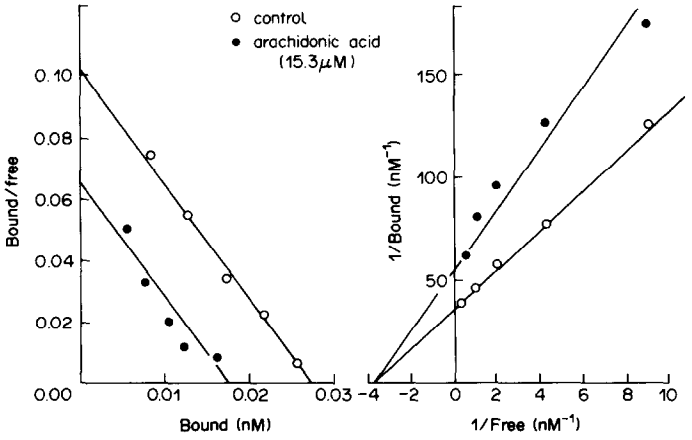

Fig. 5. Scatchard (left panel) and Lineweaver–Burk plots (right panel) on specific [^{3}H]R5020 binding to neonatal cortical cytosol receptor in the presence and absence of arachidonic acid. Each point represents the mean of two determinations from a single experiment. ○, Control: ●, arachidonic acid, 15.3 μM.

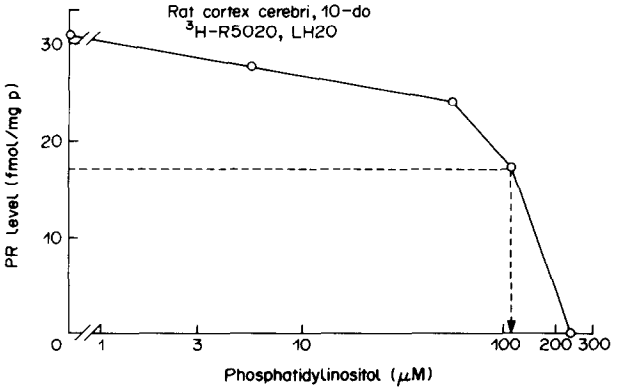

Fig. 7. The inhibition of specific [^{3}H]R5020 binding to the neonatal rat cerebral cortical cytosol progestin receptor by phosphatidylinositol (PI). The neonatal cortical cytosols (300 μg protein, 0.21 ml) were preincubated with PI (0.56–227 μM) for 15 min at 0°C, and then incubated further with [^{3}H]R5020 (4.3 nM, 87 Ci/mmol) for 4 h at 0°C. Bound [^{3}H]R5020 was separated from unbound steroid by gel filtration on LH20. Specific [^{3}H]R5020 binding was expressed as fmol/mg cytosol. Each value derives from duplicate or triplicate determinations.

As shown in Fig. 7, inhibition of specific progestin binding to the cytosol receptor was found with increasing doses of PI.

Its inhibitory effect was clearly observed at the concentration of 55.7 μM or more. PI at 226 μM completely inhibited the binding. The K_i value for PI was calculated to be approximately 111 μM.

The nature of PI inhibition of [^{3}H]R5020 binding to its brain receptor

Scatchard analysis was performed to examine the nature of PI inhibition of specific [^{3}H]R5020 binding to its receptor. The cytosol (0.21 ml, 300 μg protein) was incubated at 0°C for 15 min with or without PI (111 μM), and further incubated at 0°C for 4 h with [^{3}H]R5020 (87 Ci/mmol, 0.15–4.22 nM, on 6 levels). Specific progestin binding was separated and measured by the method of LH20.

As shown in Fig. 8 (left panel), the K_d values for progestin binding with or without PI are almost the same; 0.54 or 0.57 nM. The number of binding sites was substantially decreased in the presence of PI (19.8 fmol/mg cytosol protein) compared with that of the control (31.5 fmol/mg protein). Analysis of the same data utilizing a double reciprocal plot revealed a noncompetitive inhibition by PI (Fig. 8, right panel).

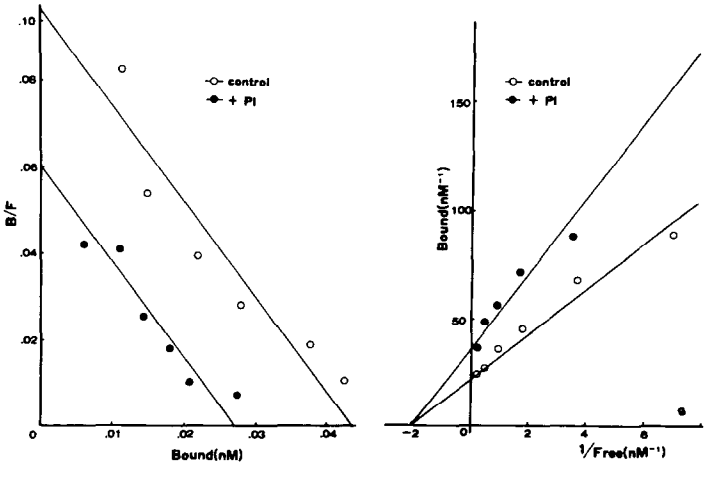

Fig. 8. Scatchard (left panel) and Lineweaver–Burk plots (right panel) of specific [^{3}H]R5020 binding to neonatal rat cerebral cortical receptor in the presence and absence of inhibitor, phosphatidylinositol (PI). Each point represents the mean of two determinations from a single experiment. Specific progestin receptor was done by LH20 method. The graded doses of [^{3}H]R5020 at multiple levels (0.15–4.22 nM) in the presence or absence of PI (20 μg/0.21 ml, 111 μM).

DISCUSSION

Results obtained from sucrose gradient and gel filtration studies in the present experiments (Figs 1 and 2) have clearly shown that long-chain unsaturated nonesterified fatty acids appear to inhibit specific progestin and dexamethasone to the respective receptors in the neonatal cerebral cortical cytosol. Saturated fatty acids had no effect. Arachidonic and docosahexaenoic acids were found to be strong inhibitors. The arachidonate inhibition was dose dependent (Figs 3 and 4). Inhibitory potency expressed by the concentration for 50% maximum inhibition (K_i) of the neonatal cortical progestin receptor by arachidonic acid was 16.5 μM (Fig. 4).

The present data on the unsaturated fatty acid inhibition on the brain progestin binding have confirmed and extended our preliminary report [14, 15]. This inhibitory effect of a long-chain unsaturated fatty acid on the brain progestin and, possibly, glucocorticoid receptors is essentially the same as that on estrogen receptor in rat uterus [5, 6], in which tissue the unsaturated fatty acid can modulate estradiol binding to its receptor. It is thus implied that the unsaturated fatty acid plays a modulatory role in the interaction of steroid hormone with its receptor in the brain.

Arachidonic and docosahexaenoic acids were found to be the strongest inhibitor of brain progestin and glucocorticoid binding in the brain (Figs 1 and 2). It is interesting to mention that variations are seen in the types of long-chain fatty acyl groups of different constituents; that is, the preponderant unsaturated fatty acyl groups of the phospholipids in nerve endings are arachidonic and docosahexaenoic acids in contrast with mainly oleate in myelin [8]. Although the brain has only trace amounts of free fatty acids, the concentrations of free unsaturated fatty acids increase rapidly in the brain under certain conditions [8]. The main increases were seen in unsaturated fatty acids, oleate and especially arachidonate [25, 26]. The fatty acids released from the membrane phospholipids may interact with progestin- and, possibly, glucocorticoid receptor in the brain to modulate its receptor.

The present experiments have demonstrated that phospholipid, phosphatidylinositol, PI, inhibits the binding of progestin to its cerebral cortical cytosol receptor with a K_i value of 111 μM (Figs 6 and 7). Inositol phospholipid, containing arachidonic acids, is the most actively metabolized of the cerebral phosphatides and is involved in certain aspects of neurotransmission, especially aorenergic and muscarinic cholinergic systems [8, 27, 28]. Moreover, according to Nishizaki *et al.*[29], signal-induced turnover of the phosphoinositides is important in the action of extracellular messengers, which appear not to be related to cyclic-AMP but Ca^{2+} dependent. In this context, it is interesting to mention the report of Chen *et al.* [30] that phospholipids and fatty acids modulate 1,25-dihydroxyvitamin D_3 receptor in chick intestinal cytosols. Our preliminary data have demonstrated the phospholipid inhibition on [^{3}H]progestin to its receptor in rat uterine cytosols (Kato *et al.*, unpublished data). The present data on the brain, together with the above-mentioned results and findings, suggest some modulation of progestin receptor in the brain cytosol by inositol phospholipid.

Specific binding of [^{3}H]R5020 to its receptor in the cortical cytosol was decreased by arachidonate with no apparent change in the binding affinity (Fig. 5). A double reciprocal plot (Lineweaver–Burk plot) has revealed that the nature of the inhibition by arachidonate is non-competitive (Fig. 5). The PI inhibition of [^{3}H]R5020 binding in the cortex has been also found to be non-competitive in nature (Fig. 8). Interestingly, 1,25-dihydroxyvitamin D_3 binding in chick intestinal cytosols is inhibited non-competitively by some phospholipids having unsaturated acyl group [30]. From kinetic and Scatchard analysis it is thus suggested that phospholipids or free fatty acids may bind at a site different from the progestin binding sites, and inhibit the steroid binding via a noncompetitive conformational change in the receptor molecule.

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