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Inhibition of Hypothalamic and Pituitary Muscarinic Receptor Binding by Progesterone

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Abstract. The in vitro effects of estrogens, progestins, and their related analogs on muscarinic receptor binding sites were studied in the hypothalamic membranes prepared from ovariectomized rats. The binding assays were performed under a nonequilibrium condition. Progestins and their metabolites were active in inhibiting the binding of [^{3}H](–)QNB to muscarinic receptors, whereas estrogenic compounds were devoid of this effect. Progesterone was also found to be active in inhibiting the binding of [^{3}H](–)QNB to pituitary membranes. The IC_{50} values of progesterone and its metabolite, 17α-hydroxyprogesterone, were 34 and 24 μM, respectively. The inhibitory effect of progesterone was rapid, reversible, and not dependent on divalent metal ions (Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}). Analyses of the binding data with Scatchard and Lineweaver-Burke plots revealed that progesterone significantly increased the apparent K_d of muscarinic receptor binding sites from 0.54 (SE = ±0.08) nM to 2.44 (SE = ±0.49) nM in hypothalamic membranes and from 0.21 (SE = ±0.03) nM to 0.34 (SE = ±0.03) nM in pituitary membranes without a significant effect on the receptor density in both membrane preparations. Progesterone decreased the rate of association of [^{3}H](–)QNB with muscarinic receptors without a significant effect on its rate of dissociation from the [^{3}H](–)QNB-receptor complex. These results indicate that progesterone, but not estrogenic compounds, was capable of interacting with hypothalamic and pituitary muscarinic receptors in vitro.

The sex hormones, estrogen and progesterone, have long been known to be involved in the regulation of the release of gonadotropin [9, 23, 29] as well as in the expression of mating behavior in female rats [1, 6]. In addition to these neuroendocrine effects, they have also been reported to have various pharmacological effects such as hypnotic [16], anticonvulsant [5, 26, 44, 47], and anesthetic [17, 32, 42, 43] effects. It is thought that various effects of sex steroids on neuronal tissues are mediated by their genomic action involving genetic transcription and subsequent protein synthesis [30], since such an action has been well established for the uterine tissue [19]. However, the sex steroids might have other direct actions at certain components of neuronal membranes resulting in some of their endocrine, behavioral, and neurological effects [31]. Evidence supporting this idea has mainly derived from the following electrophysiological studies. The sex steroids have been shown to have an almost immediate effect in altering the electrical activity of nerve cells. Such an effect is too rapid to be explained by their genomic action. Estrogen has been shown electrophysiologically to have an inhibitory effect on the firing rates of hypothalamic neurons following intravenous infusion [11, 48] or via microiontophoresis onto membranes of nerve cells [20–22]. Similarly, depression of hypothalamic neuronal activity by progesterone following intravenous infusion has also been demonstrated [25, 35]. All these previous results can be taken collectively to suggest that ovarian hormones might have a direct action on hypothalamic neuronal membranes or neurotransmitter receptors resulting in an alteration of neuronal excitability.

There are several lines of evidence implicating that the sex hormones may have one of the actions to interact with the muscarinic cholinergic system in the hypothalamus which is known to contain the centers for the expression of mating behavior of female rats [37] and for the regulation of the release of gonadotropin [45]. First, estrogen and progesterone are well known to have a synergistic action on the expression of sexual behavior of female rats [1, 6]; that

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behavior, however, can also be facilitated by muscarinic cholinergic agents and inhibited by anticholinergic agents [7, 8]. Second, in regard to the release of gonadotropin, tenuous evidence also implicates that cholinergic mechanism may mediate its release [28]. Third and finally, estradiol [24, 38] and progesterone [36, 40] have been shown to be concentrated by the hypothalamus of which the characteristics of muscarinic receptors have also been shown to be altered by hormonal manipulations of ovariectomized rats or during the estrous cycle in intact female rats [3, 13, 14, 39]. Thus, an interaction might exist between sex steroids and muscarinic receptors of the hypothalamus at the biochemical level.

Muscarinic receptors have also been found in the pituitary gland [34, 41], and their biochemical characteristics have been reported to vary with respect to the hormonal status of the animals [4]. It has also been shown that estrogen is capable of rapidly inducing a calcium-dependent action potential in pituitary cells [12]. Therefore, the possibility exists that, in addition to the hypothalamus, the pituitary gland may be another anatomical site where sex hormones and muscarinic cholinergic receptors might interact.

In view of the possible interaction between sex hormones and the cholinergic system, the present study was designed to investigate the in vitro effects of estrogen, progesterone, and their related compounds on muscarinic receptors in hypothalamus and pituitary with radioligand binding assay, and to determine the nature of such an interaction.

Materials and Methods

Chemicals

L-[Benzilic-4,4′-^{3}H]-quinuclidinyl benzilate ([^{3}H](−)QNB) (30.1 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.). Oxotremorine and all steroid compounds were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Animals

Sprague-Dawley female rats weighing 300–350 g were ovariectomized (OVX) for at least 3 weeks before experimentation.

Dissection of Hypothalami

Animals were sacrificed by decapitation and the hypothalami were dissected out from rat brains according to the procedure of Glowinski and Iversen [15]. For dissecting, the optic chiasma, the anterior commissure, the junction between the posterior hypothalamus and the mammillary bodies, and the junction between the cortex and the hypothalamus were taken as anterior, horizontal, posterior, and lateral boundaries, respectively.

Preparation of Hypothalamic and Pituitary Membranes

Membranes of hypothalami and pituitaries were prepared according to the previously described procedure of Heber et al. [18] with some modifications. Sodium potassium phosphate buffer (10 m*M*, pH 7.4) was used throughout the procedure. Hypothalami or pituitaries were homogenized (30 ml/g) in 50 ml of buffer with a polytron. The homogenates were centrifuged at 600 *g* for 10 min, and the collected supernatant was centrifuged again at 10,800 *g* for 10 min. The second centrifugation step was repeated again after resuspending the pellet in 50 ml of buffer. The pellet obtained was dispersed in buffer to yield a protein concentrations of 0.6–1 mg/ml (hypothalamic membranes) or 6–10 mg/ml (pituitary membranes).

Radioligand Binding Assay for Muscarinic Cholinergic Receptors

The [^{3}H](−)QNB binding assay was performed in a nonequilibrium condition for the experimental convenience of a shorter incubation time. The specific binding of [^{3}H](−)QNB was determined according to the procedure of Yamamura and Snyder [49] with minor modifications. Hypothalamic membranes (30–50 μg) or pituitary membranes (0.3–0.5 mg) were incubated with 60 m*M* sodium potassium phosphate buffer (pH 7.4) containing about 0.5 n*M* [^{3}H](−)QNB in a final volume of 0.25 ml. The nonspecific binding was obtained by including 0.1 m*M* oxotremorine. For saturation studies, varying concentrations (0.05–5 n*M*) of [^{3}H](−)QNB were added to the assay medium. In studies involving the hypothalamic membranes, the incubation was carried out at 25 °C for 60 min. In the case of the pituitary membranes, the incubation temperature used was 37 °C for the reason that the extent of the binding of [^{3}H](−)QNB to the pituitary membranes at 37 °C was seven times greater than that observed at 25 °C [41]. This substantially reduced the number of pituitary glands needed for experimentation. The incubation was terminated by filtration under vacuum through Whatman GF/B glass fiber filters which were then washed three times with 4 ml of ice-cold 60 m*M* sodium potassium phosphate buffer (pH 7.4). The radioactivity of the filters was determined by scintillation spectrometry.

Solubilization of Steroid Hormones

The steroid compounds were dissolved in 50% propanediol and then added to the incubation medium. The final concentation of propanediol was 4% and such a concentration was found to have less than 10% inhibition on [^{3}H](−)QNB binding. Therefore, propanediol was always added to the incubation medium as a control for steroid compounds to be tested.

Testing of Progesterone on the Rates of Association and Dissociation of [^{3}H](−)QNB to Hypothalamic Muscarinic Receptors

The method for determining the rates of association and dissociation of [^{3}H](−)QNB to hypothalamic or pituitary muscarinic receptors was carried out according to the procedure previously described by Yamamura and Snyder [49]. The rates of association and dissociation were measured at 35 °C instead of 25 °C, since it has been reported that only negligible dissociation of [^{3}H](−)QNB from the [^{3}H](−)QNB-receptor complex occurs at 25 °C [49]. For association experiments, hypothalamic membranes (62 μg protein/assay tube) or pituitary membranes (0.32 mg protein/assay tube) were incubated with 60 m*M* sodium potassium phosphate buffer (pH 7.4) containing 0.43 n*M* [^{3}H](−)QNB and 40 μ*M* progesterone. To obtain the nonspecific binding, 0.1 m*M* oxotremorine was included in the incubation medium. The mixtures were incubated for

2.5–60 min and then terminated. For determining the rate of dissociation, hypothalamic membranes were first incubated with [^{3}H](–)QNB at 35 °C for 20 min, and a solution of oxotremorine and progesterone was then added to the incubation mixtures to give a final concentration of 0.1 m*M* oxotremorine and 40 μ*M* progesterone. The reactions were terminated either immediately or after 15–90 min of further incubation in the same way as that described above for [^{3}H](–)QNB binding assay. In both experiments, the control for progesterone was done by adding a final concentration of 4% propanediol to the incubation medium.

Protein Determination

Protein was determined according to the method of Lowry et al. [27] using bovine serum albumin as a standard.

Data Analysis

The method of linear least square regression was used for plotting the Scatchard plots and for determining the rates of association and dissociation. For constructing the Lineweaver-Burke plots and displacement curves, a graphical method was used. To test the significance of the effects of progesterone on the binding and kinetic parameters, a two-tailed t test for matched pairs was used.

Results

The specific binding of [^{3}H](–)QNB to pituitary membranes was found to be about eight times less than that observed in the hypothalamic membranes. This necessitated the addition of about ten times more pituitary membranes than hypothalamic membranes in all experiments involving pituitaries. When estrogen-related analogs (17α-estradiol, 17β-estradiol, ethynylestradiol, diethylstilbestrol, estradiol 17β-acetate, β-estradiol diacetate, β-estradiol 17β-hemisuccinate, and estrone) were tested at a concentration of 100 μ*M* for their effects on the specific binding of [^{3}H](–)QNB to hypothalamic membranes, they were found to have no effect (data not shown). However, progestins and their related metabolites were found to be active in displacing the [^{3}H](–)QNB from the hypothalamic muscarinic receptors (fig. 1). As shown in figure 1, the progesterone metabolite, 17α-hydroxyprogesterone, was found to be the most potent inhibitor ($IC_{50} = 24$ μ*M*) among the compounds tested, and slightly more potent than progesterone ($IC_{50} = 34$ μ*M*). In addition, the IC_{50} value (82 μ*M*) of progesterone in inhibiting the binding [^{3}H](–)QNB to pituitary membranes was two times greater than that observed in the hypothalamic membranes (fig. 1). It was also observed that washing of the membranes which had been preincubated with progesterone (40 μ*M*) for 30 min completely reversed the inhibitory effect of progesterone (data not shown). Furthermore, the inhibitory effect of progesterone appeared to be very rapid and did not require preincubation (data not shown).

In an attempt to search for the optimal condition for progesterone to inhibit [^{3}H](–)QNB binding, various divalent metal ions were added to the incubation medium to test for the divalent metal ions requirement. It was found that Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, and EDTA, all at a concentration of 1 m*M*, had no effect on the inhibition of [^{3}H](–)QNB binding by progesterone. In addition, all these compounds by themselves had no effect on [^{3}H](–)QNB binding (data not shown). At a concentration of 1 m*M*, the other two divalent metal ions, Cu^{2+} and Fe^{2+}, were found to inhibit [^{3}H](–)QNB binding, without the presence of progesterone, by 44 and 34%, respectively. Therefore, it was observed that there was a greater inhibition of [^{3}H](–)QNB binding by progesterone in the presence of Cu^{2+} or Fe^{2+} (data not shown). After correcting the inhibitory effects due to these metal ions themselves, they were found to have no further effect on the inhibition of [^{3}H](–)QNB binding by progesterone.

Since estrogen had no effect on [^{3}H](–)QNB binding to hypothalamic membranes and norethynodrel was the least active inhibitor among the progesterone-related compounds tested in figure 1, an attempt was made to test whether they could enhance or block the inhibitory effect of progesterone (at a concentration of 20 μ*M*) on [^{3}H](–)QNB binding. Norethynodrel and 17β-estradiol at a concentration of 100 μ*M* were found to have no effect (data not

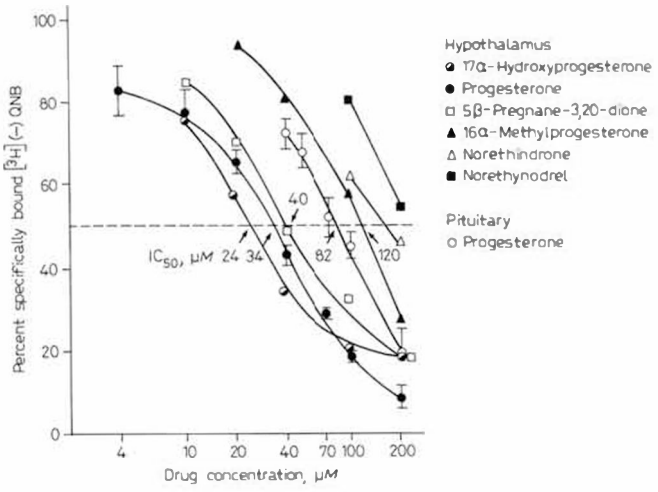

Fig. 1. The inhibition of specific [^{3}H](–)QNB binding by varying concentrations of progesterone and its related compounds. The specific [^{3}H](–)QNB binding in the absence of steroid compounds was 502 ± 13 fmol/mg protein and the concentration of [^{3}H](–)QNB in the incubation medium was 0.5 n*M*. The binding assays were performed as described in the Materials and Methods section. The values for the IC_{50} are the concentrations which inhibited 50% of the binding. The data represent means ± SE of 6–9 determinations from 2–3 separate experiments. The inhibition curves were drawn with a graphical method. Similar IC_{50} values were obtained from three other separate experiments and the standard errors were less than 10% of the values presented in the figure.

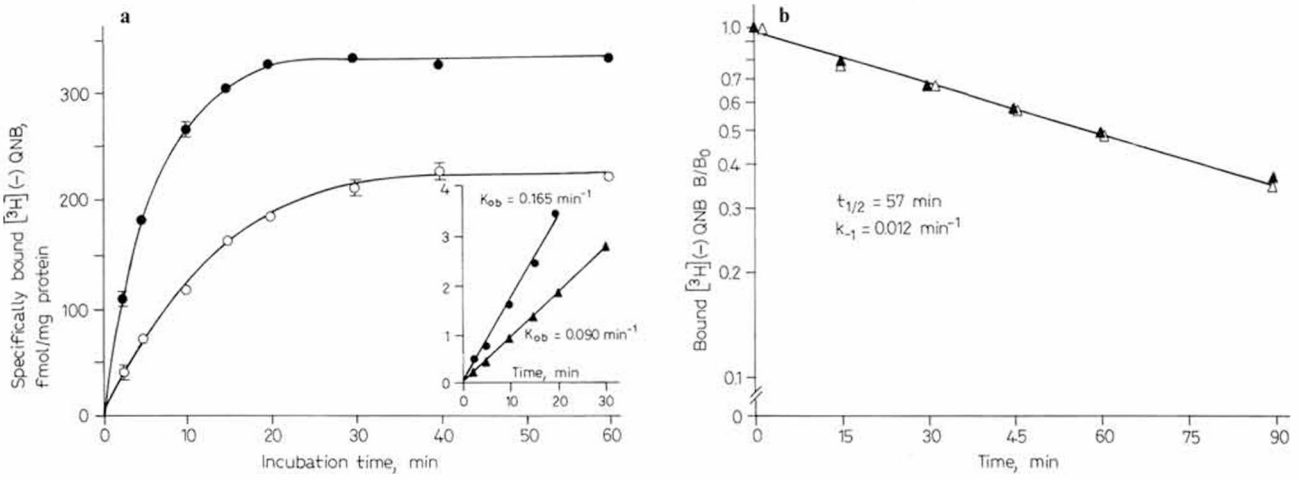

Fig. 2. a The time dependence of specific binding of [^{3}H](−)QNB to hypothalamic membranes incubated with (●) or without (○ or ▲) the addition of 40 μ*M* of progesterone to the incubation medium. The experiment was performed as described in the Materials and Methods section. The data represent means ± SE of 3 determinations. A plot of $\ln(B_e/B_e\text{-}B)$ versus time is shown in the insert where B_e = the equilibrium concentration of [^{3}H](−)QNB, B = the concentration of bound [^{3}H](−)QNB, and k_{ob} = the slope of the plot of $\ln(B_e/[B_e\text{-}B])$ versus time. The rate of association (k_1) was derived from the equation, $k_1 = (k_{ob}\text{-}k_{-1})/$free [^{3}H](−)QNB concentration. **b** The rate of dissociation of [^{3}H](−)QNB from [^{3}H](−)QNB-receptor complex in the medium containing 0.1 m*M* oxotremorine (▲) or containing both 0.1 m*M* oxotremorine and 40 μ*M* progesterone (△). The experiment was performed as described in the Materials and Methods section. The data obtained under the two conditions were almost identical. The result of the logarithmic analysis of the data of the control group is given in the figure where B = [^{3}H](−)QNB at time t, B_0 = [^{3}H](−)QNB binding at time 0, $t_{½}$ = the time at which B = 0.5 B_0, and $k_{-1} = 0.693/t_{½}$. The data points represent means of 3 determinations with standard errors of less than 5% of the means.

shown). For understanding further the mechanism by which progesterone inhibited [^{3}H](−)QNB binding, the effects of progesterone (40 μ*M*) on the rate of association of [^{3}H](−)QNB with muscarinic receptors and the rate of dissociation of [^{3}H](−)QNB from the [^{3}H](−)QNB-receptor complex were studied. The results of the experiments performed at 35 °C are shown in figure 2. Statistical analysis of the kinetic data revealed that progesterone significantly decreased the rate of association without a significant effect on the rate of dissociation in both hypothalamic and pituitary membranes (table 1). Calculation of the kinetic dissociation constant (K_d) from these two parameters indicated that progesterone caused approximately a twofold increase in the kinetic K_d values in both membrane preparations (table 1).

In order to understand whether progesterone has an effect on the affinity of the binding sites or the receptor density, saturation studies were performed on hypothalamic and pituitary membranes with and without the presence of progesterone (40 μ*M*) as described in the Materials and Methods section. For these studies, nonequilibrium condition was adopted for the experimental convenience of a shorter incubation time, since progesterone had been found to have no effect on the rate of dissociation as noted above, and the apparent K_d ($K_{d,\,app}$) values obtained in such a condition were simply a reflection of the change in the rate of association [2]. Scatchard analysis of the binding data indicated that progesterone significantly increased the $K_{d,\,app}$ without a significant effect on the receptor density (B_{max}) in both hypothalamic (fig. 3a, table 1) and pituitary (fig. 4a, table 1) membranes. The muscarinic receptor density of the pituitary membranes was found to be nine times less than that of the hypothalamic membranes. A further analysis of the binding data with Lineweaver-Burke plots revealed that progesterone significantly increased the apparent Michaelis-Menten constant ($K_{m,\,app}$) without causing a significant change in the maximum binding values in both hypothalamic (fig. 3b, table 1) and pituitary (fig. 4b, table 1) membranes.

Discussion

The present study examined the effect of estrogenic and progesterone-related compounds on the binding of [^{3}H](−)QNB to muscarinic receptors from hypothalamus and pituitary. The results indicated that (a) progesterone-related but not estrogenic compounds inhibited the binding of the muscarinic antagonist, [^{3}H](−)QNB, to muscarinic receptors; (b) the inhibition did not require divalent metal ions and was rapid and reversible; and (c) progesterone decreased the rate of association of [^{3}H](−)QNB with muscari-

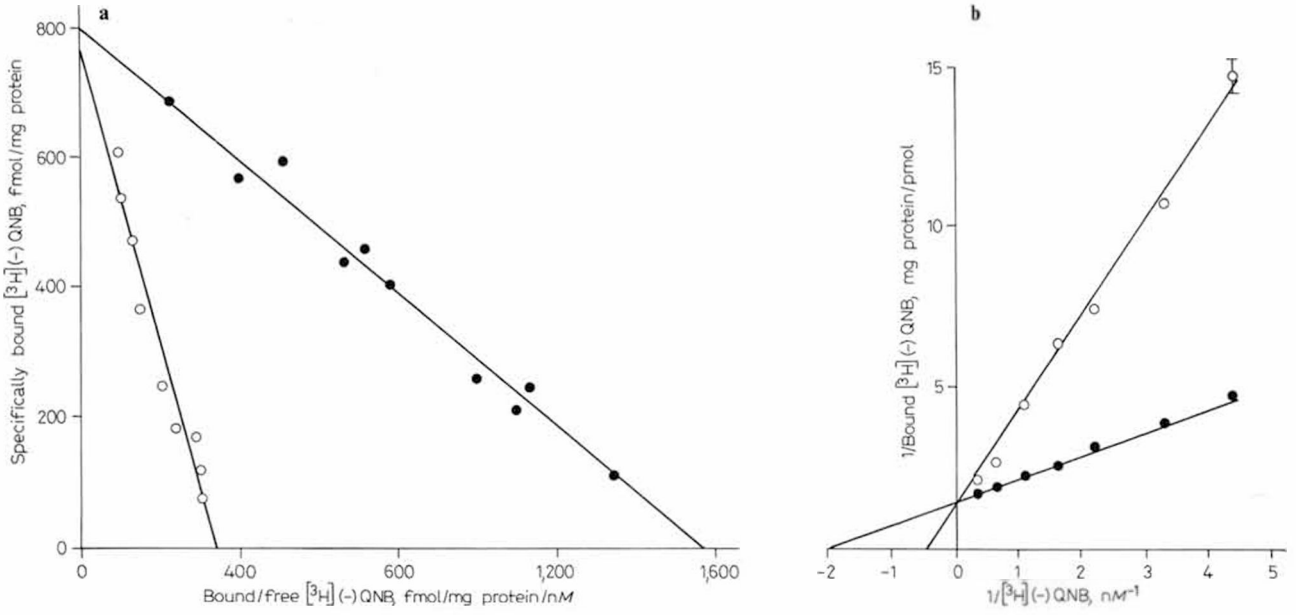

Fig. 3. a A Scatchard analysis of [^{3}H](−)QNB binding data obtained from hypothalamic membranes incubated with (○) or without (●) the addition of 40 μ*M* of progesterone to the incubation medium. The negative slope, determined by linear regression analysis, is equal to $K_{d,\ app}$. The number of binding sites, B_{max}, is equal to the intercept of the plot with the Y-axis. The data points represent means of 6 determinations from 2 experiments. **b** A Lineweaver-Burke plot of [^{3}H](−)QNB binding data from hypothalamic membranes incubated with (○) or without (●) the addition of 40 μ*M* progesterone to the incubation medium. The data represent means ± SE of 3 determinations.

Table I. Effects of progesterone on the binding and the kinetic parameters of hypothalamic and pituitary membranes

	Scatchard plot		Lineweaver-Burke plot		Kinetic properties		
	$K_{d,\ app}$ n*M*	B_{max} fmol/mg protein	$K_{m,\ app}$ n*M*	B_{max} fmol/mg protein	k_1 $nM^{-1}\ min^{-1}$	k_{-1} min^{-1}	k_{-1}/k_1 p*M*
Hypothalamic membranes							
Control	0.54 ± 0.08	755 ± 113	0.49 ± 0.10	715 ± 107	0.35 ± 0.06	0.012 ± 0.002	34 ± 6
Progesterone (40 μ*M*)	2.44 ± 0.49[1]	830 ± 124	2.15 ± 0.37[1]	732 ± 146	0.19 ± 0.04[1]	0.013 ± 0.002	70 ± 12[1]
Pituitary membranes							
Control	0.21 ± 0.03	86 ± 10	0.21 ± 0.01	91 ± 7	0.36 ± 0.07[2]	0.013 ± 0.002[2]	36 ± 5[2]
Progesterone (40 μ*M*)	0.34 ± 0.03[1]	94 ± 9	0.35 ± 0.04[1]	87 ± 8	0.18 ± 0.04[1,2]	0.012 ± 0.002[2]	68 ± 14[1,2]

The experimental and the corresponding control groups were performed in the same experiment, which was replicated four times. The data are presented with standard errors of respective means of four separate experiments. The incubation temperatures for saturation studies of the hypothalamic and the pituitary membranes were 25 and 37 °C, respectively. The incubation temperature for the kinetic studies was 35 °C.

[1] Indicates $p < 0.05$ when compared with the corresponding control group in the same column and from the same tissue source, as determined by two-tailed t test for matched pairs.

[2] Indicates no significant difference ($p > 0.05$) when compared to the corresponding kinetic parameter determined in the hypothalamic membranes.

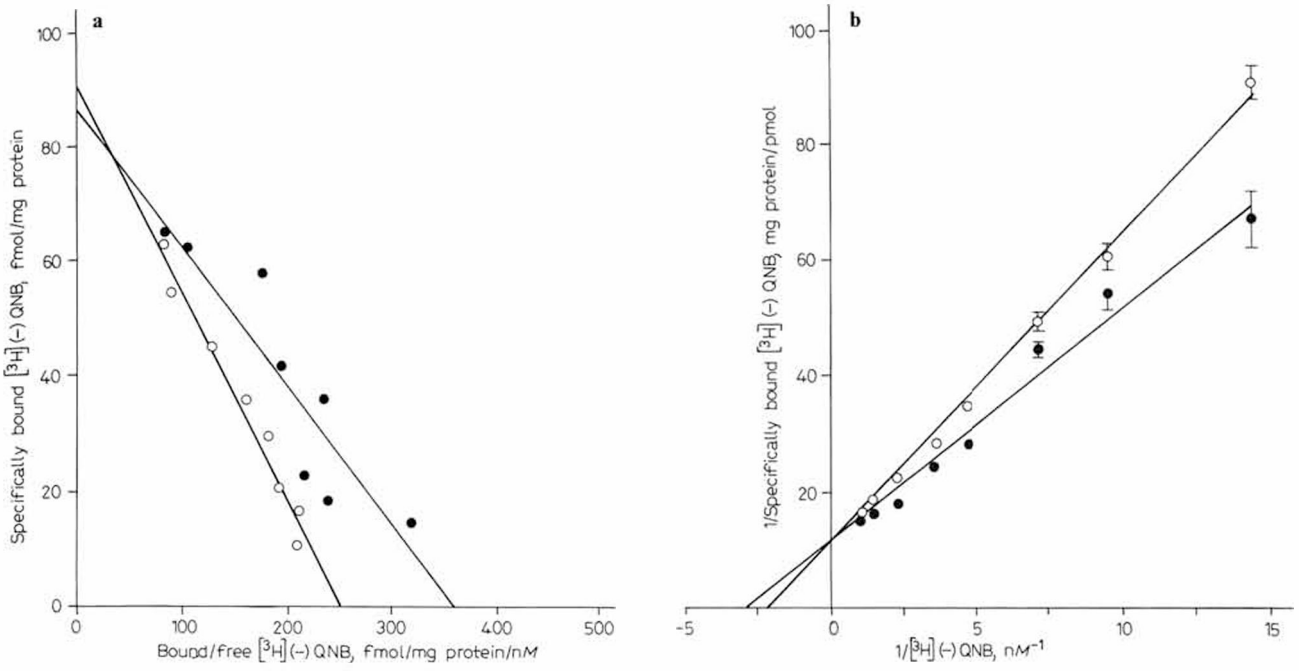

Fig. 4. **a** A Scatchard analysis of [^{3}H](−)QNB binding data obtained from hypothalamic membranes incubated with (○) or without (●) the addition of 40 μ*M* progesterone to the incubation medium. See legend of figure 2 for obtaining the values for the parameters, $K_{d,\,app}$ and B_{max}. The data points represent means of 9 determinations from 3 experiments. **b** A Lineweaver-Burke plot of [^{3}H](−)QNB binding data to pituitary membranes incubated with (○) or without (●) the addition of 40 μ*M* progesterone to the incubation medium. The data represent means ± SE of 3 determinations.

nic receptors but had no effect on its rate of dissociation from the [^{3}H](−)QNB-receptor complex.

It should be noted that there is no structural resemblance between progesterone and acetylcholine, yet progesterone was found to be capable of interacting with muscarinic receptors with a certain structural specificity. By examining the structures of all the compounds tested in figure 1, it may be concluded that the keto group at the C 3 position of the steroidal ring is essential for the compound to interact with the muscarinic receptor. This might provide the explanation as to why all estrogenic compounds tested, possessing a hydroxy instead of a keto group at the C 3 position, were virtually devoid of this effect. This idea is further supported by the finding that 17β-estradiol failed to block the inhibitory effect of progesterone on [^{3}H](−)QNB binding.

The findings that progesterone inhibited [^{3}H](−)QNB binding in a reversible manner, and that estrogen was devoid of this effect are consistent with the previous observation that sex differences and different stages of the estrous cycle did not influence the binding of muscarinic antagonist to membranes prepared from the whole hypothalamus [3], since the direct effect of progesterone exerted in vivo, if any, would be lost during preparation of the membranes. Although estrogen was found to have no in vitro effect on [^{3}H](−)QNB binding in this study, it has been reported that administration of estrogen to ovariectomized rats resulted in an alteration of the receptor density of muscarinic receptors [10, 39]. This previous result suggests that estrogen may have an in vivo action to alter the number of muscarinic receptors through a genomic action or other unknown mechanisms.

It has been reported that high affinity binding sites of progesterone with a dissociation constant of 10^{-8} *M* are present in synaptic membranes prepared from rat brains, and that the bound progesterone can be displaced by progesterone itself but not by a 500-fold excess of 17β-estradiol [46]. In view of the present finding that 17β-estradiol, unlike progesterone, had no effect in displacing [^{3}H](−)QNB from the muscarinic receptor, it would appear that there is a parallelism between the displacement of progesterone from its binding sites and the displacement of [^{3}H](−)QNB from muscarinic receptors by estrogen and progesterone. Thus, it is possible that muscarinic receptors and the binding sites for progesterone are the same sites in the synaptic membranes. However, a meaningful comparison between the K_d value of the progesterone binding sites and the K_i value for progesterone to inhibit [^{3}H](−)QNB binding cannot be

made in this study for the following two reasons. First, the membrane preparations used in this study were substantially less pure than the ones employed by the previous investigators [46]. The purity of the membrane preparations can affect the effective concentration of progesterone in the medium to inhibit [^{3}H](–)QNB binding, since progesterone possessing lipophilic property can bind to nonreceptor materials and other cellular debris. Second, the displacement curve (fig. 1) for progesterone to inhibit [^{3}H](–)QNB binding was obtained in a nonequilibrium condition in this study and the true K_i value has to be obtained with a curve-fitting algorithm technique [33]. Therefore, the confirmation that the binding site for progesterone and the muscarinic receptor are the same entity in the synaptic membrane must await for the determination of the K_d value of progesterone binding sites and the proper determination of the K_i value for progesterone in the same highly purified membrane preparation.

Analyses of the binding data with Scatchard plots and Lineweaver-Burke plots (table I) suggest that the inhibition of [^{3}H](–)QNB binding by progesterone was competitive in nature, since progesterone caused a change in the binding affinity without affecting the receptor density (table I). However, since a complete inhibition by progesterone could not be shown in this study due to the partial insolubility of the steroidal hormones at concentrations greater than 200 μM, the possibility that progesterone interacts with an allosteric site cannot be excluded. Therefore, progesterone could decrease the rate of association of [^{3}H](–)QNB with muscarinic receptors in vitro by competing for the occupancy of muscarinic receptors or by interacting with an allosteric site to cause a decrease in the affinity of muscarinic receptors for [^{3}H](–)QNB. The lack of effect of progesterone on the rate of dissociation suggests that progesterone has no effect on [^{3}H](–)QNB-receptor complex, once the binding domain has already been occupied by [^{3}H](–)QNB. The shapes of the binding curves in the absence and in the presence of progesterone as a function of time as depicted in figure 2a is very similar to that of a theoretical plot for a competitor which dissociates more rapidly than does the radioligand from the receptors [33]. This is in agreement with our initial observation that the inhibitory effect of progesterone did not require preincubation and was rapidly reversible, whereas the dissociation of [^{3}H](–)QNB from the receptors was very slow ($k_{-1} = 0.012$ min^{-1}).

On the basis of the data presented in table I, there appears a similarity between the hypothalamic and the pituitary [^{3}H](–)QNB binding sites, since there was no significant difference between the two membrane preparations in regard to the values of k_1, k_{-1}, and the kinetic K_d as well as the extent of the decrease in K_d by progesterone. When the hypothalamic membranes were tested with progesterone at 25 °C, the decrease in the $K_{d,\,app}$ was more pronounced than that observed in the pituitary membranes incubated at 37 °C. This is simply due to the fact that the experiments performed at 25 °C deviated more from equilibrium condition than those performed at 37 °C resulting in a greater decrease in the $K_{d,\,app}$ value. The finding that the interaction between progesterone and the muscarinic receptor was very rapid may provide an explanation for the previous electrophysiological observations demonstrating the short latency (20 min) and the short duration of action (1 h) of progesterone in altering neuronal activity following intravenous administration [25, 35]. Since progesterone's metabolite, 17α-hydroxyprogesterone, was found to be more potent than progesterone in inhibiting the binding of [^{3}H](–)QNB to muscarinic receptors, other more potent metabolites might exist. A search for more potent metabolites might be important in understanding the modulation of the cholinergic system by progesterone. Since the hypothalamus preferentially concentrates progesterone [36, 40], the combined effects of progesterone and its other metabolites on muscarinic receptors might have pharmacological importance with progestin being a component of oral contraceptives. Their physiological importance in relation to progesterone's neuroendocrine function remains as yet to be elucidated.

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