Effects of neuroactive steroid hormones on learning and memory

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Abstract

Here we describe effects of neuroactive steroids, estrogen and progesterone, on cognitive functions. These neuroactive steroids are synthesized in the central and peripheral nervous system including other tissues. They are involved in the regulation of learning and memory, or mood formation in premenstrual syndrome, and these are related to hormone replacement therapy in addition to postnatal and major depression, anxiety disorders, and Alzheimer's disease. Estrogen and progesterone have their individual receptors. The action of estrogen and progesterone can be direct genomic, indirect genomic, or non-genomic, also influencing several neurotransmitter systems. Estrogen has been related to improved learning and memory, or mood formation. On the other hand, progesterone may have negative effects. In this chapter we introduce following our three experimental results. 1) Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze. Here we suggested that OVX or low-calcium diet might impair learning and memory and that the combination of these factors impaired more markedly, and these also implied the possibility that a woman in menopause or post-menopause might suffer impairment of learning and/or memory when intakes low-calcium diet[1]. 2) Effects of estradiol and progesterone on radial maze performance in rats fed a low-calcium diet. Here we suggested the possibility that treatment with estradiol under low-calcium conditions could not improve impaired learning and memory when progesterone was applied simultaneously, and that the intake of adequate calcium might be necessary and effective for patients with learning and memory hypofunction receiving hormone replacement therapy[2]. 3) Effects of steroid hormones on (Na^+, K^+)-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task. Here we indicated that estrdiol and testosterone ameliorated the amnesia induced by inhibition of (Na^+, K^+)-ATPase activity, and that the protective effect of estradiol might be caused by a non-genomic, rather than a genomic effect or a radical scavenging action. Additionally, the ameliorative effect of testosterone did not appear to involve free radical scavenging, but its aromatization to estrogen could contribute to the non-genomic action of estradiol[3].

Key words: learning and memory, Estradiol, Progesterone, Neuroactive steroids, calcium deficiency, Morris water maze, passive avoidance

Neuroactive steroids

Neuroactive steroids, estradiol and progesterone, are synthesized in the central and peripheral nervous system including neurons and astrocytes[4, 5]. The precursor, cholesterol, is supplied or derived from low-density lipoproteins in many cells including the nervous system[6, 7, 8]. The cytochrome P450 side-chain cleavage enzyme (P450scc) is related to the conversion of cholesterol to pregnenolone[9]. Pregnenolone is oxidized to progesterone by the 3β-hydroxysteroid dehydrogenase/isomerase. Estradiol is converted from testosterone in the peripheral or the brain by aromatase P450. This enzyme is localized in hippocampal neurons-pyramidal cells of the CA1-CA3 regions in addition to the granule cells of the dental gyrus[10]. The central nervous system is able to take up the steroids from the blood. These indicate that the brain concentrations are related to the peripheral production in the endocrine organs. Estradiol and progesterone are the major human female sex hormones. In the adult women, the main sources of estradiol are the granulosa cells of the developing follicle and the corpus luteum[11]. The adrenals can also produce androstenedione, which is converted to estrone and estradiol, or to testosterone as the brain can produce and convert to these steroids.

Progesterone is mainly synthesized in granulosa cells of the corpus luteum as well as in the placenta and the adrenals[11]. Following synthesis, most of the estradiol and progesterone are bound to plasma proteins such as globulin, albumin, transcortin though bounding hormones are relatively inactive.

Estrogens are required for the normal female phenotype, female genital function, sexual maturation but also for skeleton maintenance and are protective for the cardiovascular system[12] as well as for the central nervous system. Progesterone is a very important hormone for conception and pregnancy maintenance. Ovarian steroids have important effects on brain function, including learning and memory, regulation of the reproductive neuroendocrine system, or mood formation[13, 14]. As neuroactive steroids are lipophilic and relatively small molecules, they readily across the blood brain barrier and become available for their actions on the central nervous system. Neuroactive steroid concentrations in plasma and the brain vary throughout the menstrual cycle and decrease in woman in menopause or post-menopause.

Neuroactive steroid receptors

Estrogen receptors (ER; ERα and ERβ) and progesterone receptors (PR; PRα and PRβ) belong to a super family of transcription factors, the nuclear receptor family[15, 16]. ERs consist of individual domains such as N-terminal domain, DNA-binding domain, hinge ligand binding domain, and C-terminal domain[17]. These diffuse into the cell and bind to their own receptors with transformation or activation of the receptors. Activation comes from dissociation of the receptor-heat shock protein complex and formed dimerarization. The dimer binds to specific DNA hormone response elements in the promoter region of target genes and initiates transcription, subsequently leading to translation[11]. ERα and ERβ can form both homo- or heterodimers[18, 19] as with PRα and PRβ[20]. Phosphorylation sites in these receptors have been identified, and DNA binding and transcription are modified by phosphorylation. Receptors are phosphorylated in the absence of ligands and exhibit further phosphorylation on ligand binding[21]. Estrogen receptors also be able to regulate transcription through binding to the AP-1 response element[22] and are distributed in many organs such as the uterus, ovaries, lungs, breast, and the central nervous system[23, 24]. In the central nervous system, they are localized in the hippocampus, cortex, amygdala, the septum, and the hypothalamus[25, 26, 27, 28].

Progesterone receptors are also distributed in many tissues including the central nervous system[29, 30, 31]. The action of neuroactive steroid hormones could be direct genomic, indirect genomic or non-genomic[32].

The direct genomic mechanism of estrogen involves the association of the estrogen-ER dimer complex with estrogen response element or with the fos/jun heterodimers bound to activator protein 1. The indirect genomic mechanism involves activation of ERs linked to the second messenger systems such as protein kinase A or C, mitogen-activated protein kinase (MAPK), extracellular signal regulated kinase (ERK), cAMP response element binding protein (CREB) and nuclear factor-κB. Non-genomic effects are the stabilization of the mitochondrial membranes and reduction of the generation or the scavenging of free oxygen radicals, with a resultant neuroprotective effect[33, 34, 35].

Progesterone also has a direct genomic mechanistic action onto its receptor, coupled to co-activators, CREB

binding protein and an indirect genomic action is through GTP binding protein.

Action of steroid hormones also seems to be through neurotransmitter systems including the cholinergic, serotonergic and GABA ergic systems[36, 37, 38]. Furthermore, estradiol induces NMDA receptor expression in the CA1 region of the hippocampus and NMDA receptor antagonists block estrogen-induced synaptogenesis on dendritic spines[38, 39].

Experimental results and their outline on learning and memory

1) Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze in middle-aged female rats[1)]

To examine the effect of estrogen deficiency and low-calcium diet on learning and memory, middle-aged female Wistar rats (50 weeks old) were fed either a low-calcium (0.02% Ca) or a normal-calcium (1.25% Ca) diet throughout the experiment. Rats were ovariectomized (OVX) or sham-operated (Sham). These animals were divided into four groups: 1) Sham group with normal-calcium diet [Sham-normal Ca group], 2) OVX group with normal-calcium diet [OVX-normal Ca group], 3) Sham group with low-calcium diet [Sham-low Ca group], 4) OVX group with low-calcium diet [OVX-low Ca group]. Seventy-seven days after the OVX or Sham operation, the learning and memory abilities in the female rats were examined by using a radial maze task according to the method of Olton and Samuelson (regular trials) and using a delay-interposed task following regular trials. During regular trials and delay-interposed tasks, the OVX-low Ca group was inferior to all the other groups in accuracy of choice behavior. Both Sham-normal Ca and Sham-low Ca groups showed more accurate choices than the OVX-low Ca group, but were less accurate than the Sham-normal Ca group. In addition, there was no significant difference in locomotor activity between any of the groups. These results suggest that OVX or low-calcium diet may impair learning and that the combination of these factors impaired more markedly when the rats were tested in the eight-arm radial maze. These results may also imply the possibility that a woman in menopause or post-menopause suffers impairment of learning and/or memory when intakes low-calcium diet.

Above abstract was derived mainly from the following experimental results.

Fig. 1 plots number correct until first mistake (Fig. 1A) and total errors (Fig. 1B) in blocks of six trials. In Fig. 1A, a significant effect for each treatment group is observed (F (3,24)=11.93, P<0.01), relative to trial block (F (4,12)=42.59, P<0.01) and interaction between treatment groups in the trial blocks (F (4,96)=5.95, P<0.01). These measurements indicate that the change in the number correct until the first mistake for each treatment group was different during the regular trials (i.e. each treatment group was different in its ability to resolve our radial maze task) and that these subjects, except for the OVX-low Ca group, showed an increase in the number of correct choices as the trials progressed. Furthermore, we also compared each treatment group by contrast analysis. There were significant difference between the Sham-normal Ca and OVX-normal Ca (P < 0.01), Sham-normal Ca and OVX-low Ca (P <0.01), Sham-low Ca and OVX-low Ca (P<0.01), and OVX-normal Ca and OVX-low Ca (P<0.05) groups. In addition, a comparison by contrast analysis also indicated a significant interaction of the treatments in the trial blocks between Sham-normal Ca and OVX-normal Ca (P<0.01), Sham-normal Ca and Sham-low Ca (P<0.05), Sham-normal Ca and OVX-low Ca (P<0.01), Sham-low Ca and OVX-low Ca (P<0.01), and OVX-normal Ca and OVX-low Ca (P<0.01) groups. Fig. 1B demonstrates the significant effect of each treatment group (F (3,24)=9.96, P <0.01), trial block (F (4,12)=25.01, P<0.01), and interaction between treatment groups in the trial blocks (F (4,96)=2.85, P<0.01). The results of this analysis also revealed that the change in the total errors for each treatment group was different and that in the treated subjects, total errors decreased as the trials progressed. The comparisons by contrast analysis indicated significant differences between Sham-normal Ca and Sham-low Ca (P<0.05), Sham-normal Ca and OVX-low Ca (P<0.01), OVX-normal Ca and OVX-low Ca (P<0.01), and Sham-low Ca and OVX-low Ca (P<0.01) groups. Moreover, contrast analysis for total errors also revealed significant interactions of the treatments in the trial blocks between Sham-normal Ca and OVX-low Ca (P<0.01), OVX-normal Ca and OVX-low Ca (P<0.01), and Sham-low Ca and OVX-low Ca (P<0.01) groups.

Fig. 2 shows the number correct until the first mistake

(A) and the total errors (B) over the 12 delay-interposed radial maze tasks (delay trials). Each time-delay period is the mean of three consecutive delay trials. For the number correct until the first mistake, there were significant treatment effects ($P<0.01$) at all interposed delay times: 30 min, F (3,24)=11.97; 1 h, F (3,24)=7.33; 2 h, F (3,24)=5.25; 3 h, F (3,24)=9.15. Post-hoc tests indicated that rats in the OVX-normal Ca group had lower scores than those in the Sham-normal Ca group at 30 min ($P<0.05$) and 3 h ($P<0.05$), and that Sham-low Ca-group rats also showed lower scores than the Sham-normal Ca group at 30 min ($P<0.01$) and 2 h ($P<0.05$). Furthermore, OVX-low Ca-group rats showed lower scores than all the other groups at each interposed delay time. In short, the post-hoc test indicated that OVX-low Ca-group rats had lower scores than not only the OVX-normal Ca group (30 min, $P<0.05$; 1 h, $P<0.01$; 2 h, $P<0.05$; 3 h, $P<0.01$) but also Sham-low Ca-group rats (1 h, $P<0.05$; 3 h, $P<0.01$). For the total errors, there were significant differences ($P<0.01$) between the four treatment groups at all interposed delay times: 30 min, F (3,24)=6.57; 1 h, F (3,24)=15.07; 2 h, F (3,24)=14.54; 3 h, F (3,24)=5.24. The post-hoc tests indicated that there was no significant difference between the OVX-normal Ca and Sham-normal Ca groups but a trend of increasing total errors was observed in the OVX-normal Ca group, and that total errors for the Sham-low Ca and OVX-low Ca groups were significantly higher than for the Sham-normal Ca group ($P<0.01$), but there was no significant difference between the Sham-low Ca and OVX-low Ca groups. In addition, OVX-low Ca-group rats showed a significantly increased value for total errors compared to the OVX-normal Ca-group rats at each interposed delay time (30 min, $P<0.05$; 1 h, $P<0.01$;

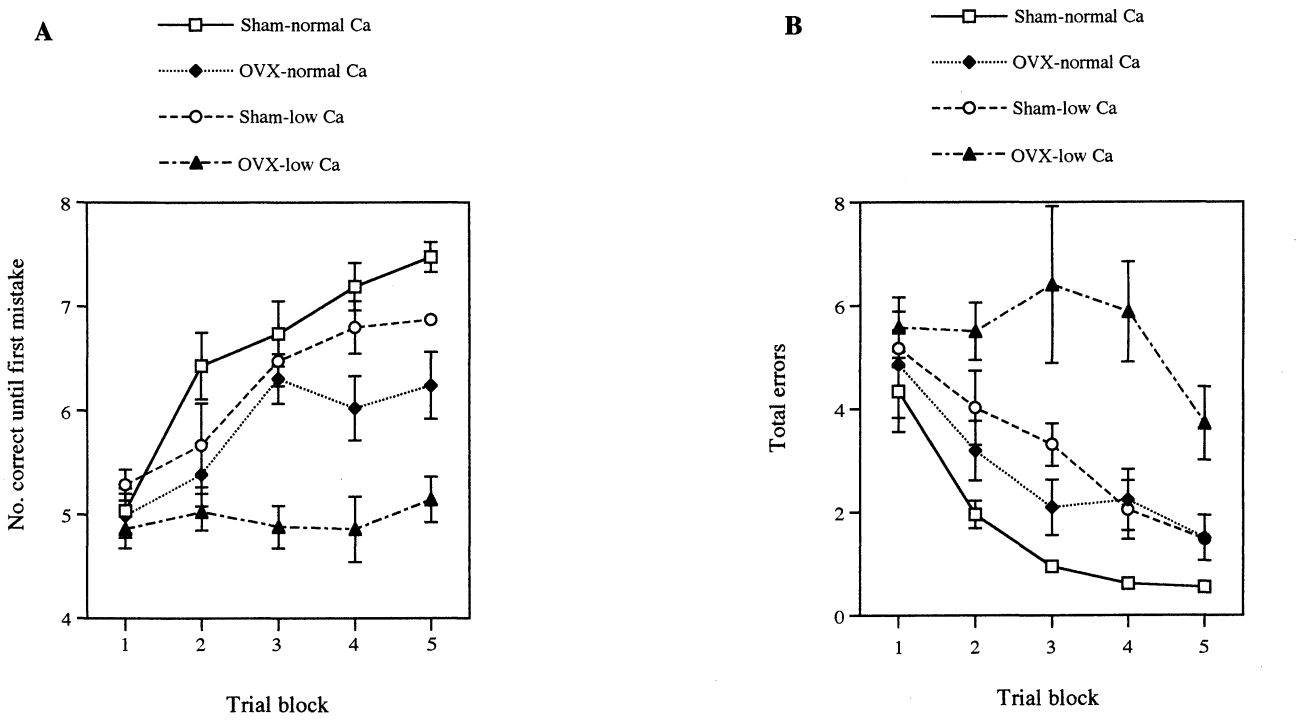

Fig. 1. Changes in the number of correct choices until the first mistake (A) and total number of incorrect choices (B) for maze performance of each treatment group (Sham-normal Ca: normal control diet plus sham-ovariectomy (□), OVX-normal Ca: normal control diet plus ovariectomy (◆), Sham-low Ca: low-calcium diet plus sham-ovariectomy (○), OVX-low Ca: low-calcium diet plus ovariectomy (▲)). Values are the average number of correct choices until the first mistake±S.E.M. (A) and the average total number of error choices±S.E.M. (B) for seven rats per group. A block is the mean of six consecutive trials. Results are expressed as the mean±S.E.M. Choice accuracy data for regular trials were averaged to give blocks of six trials each. The differences in choice accuracy parameters in the regular trials were analyzed by repeated-measures ANOVA, and comparisons of changes between the treatment groups were made by contrast analysis. All the other data among groups were analyzed by one-way analysis of variance (ANOVA) with post-hoc tests (Fisher's partial least square difference (PLSD)). Statistical significance was defined as $P<0.05$. Data analyses were performed using Super ANOVA 1.11.

2 h, $P<0.01$; 3h $P<0.01$).

2) Effects of estradiol and progesterone on radial maze performance in middle-aged female rats fed a low-calcium diet[2].

To examine the effect of ovarian steroids on learning and under a low-calcium condition, middle-aged female rats were fed either a low-calcium (0.02% Ca) or a normal-calcium (1.25% Ca) diet. All rats were ovariectomized (OVX), and these animals were divided into eight groups: 1) an OVX group with a normal-calcium diet (OVX-normal-Ca group), 2) an OVX group with 17β-estradiol treatment and a normal-calcium diet (E2 group), 3) an OVX with progesterone treatment and a normal-calcium diet (P4 group), 4) an OVX with 17β-estradiol and progesterone treatments and a normal-calcium diet (E2+P4 group), 5) an OVX group with a low-calcium diet (OVX-low-Ca group), 6) an OVX group with 17β-estradiol treatment and a low-calcium diet (LE2 group), 7) an OVX group with progesterone treatment and a low-calcium diet (LP4 group), and 8) an OVX group with 17β-estradiol and progesterone treatments and a low-calcium diet (LE2+LP4 group). Seventy-seven days after the OVX operation, the learning and memory abilities of the rats were examined by using an eight-arm radial maze task. E2 and E2+P4 groups learned in fewer trials, and performed better in the radial maze and the working memory task than the other groups under the normal-calcium condition. Rats in the LE2 group learned in fewer trials, and performed better in the maze and working task than the other low-calcium groups, but in combination with progesterone under the low-calcium condition (LE2+LP4 group), the facilitative effect of estradiol in all the tasks was inhibited. Treatment with progesterone alone did not inhibit the learning and memory task performance. These results suggest the possibility that treatment with estradiol under low-calcium conditions cannot improve impaired learning and memory when progesterone is applied simultaneously, and that the intake of adequate calcium may be necessary and effective for patients with learning and hypofunction receiving hormone replacement therapy.

Above abstract was derived mainly from following experimental results[2].

Fig. 3 shows the number of trials until the criterion was reached (trials to criterion), the number of correct choices until the first mistake (number correct until first mistake) and the total number of incorrect choices per trial (total errors) in each diet and treatment group. Trials to cri-

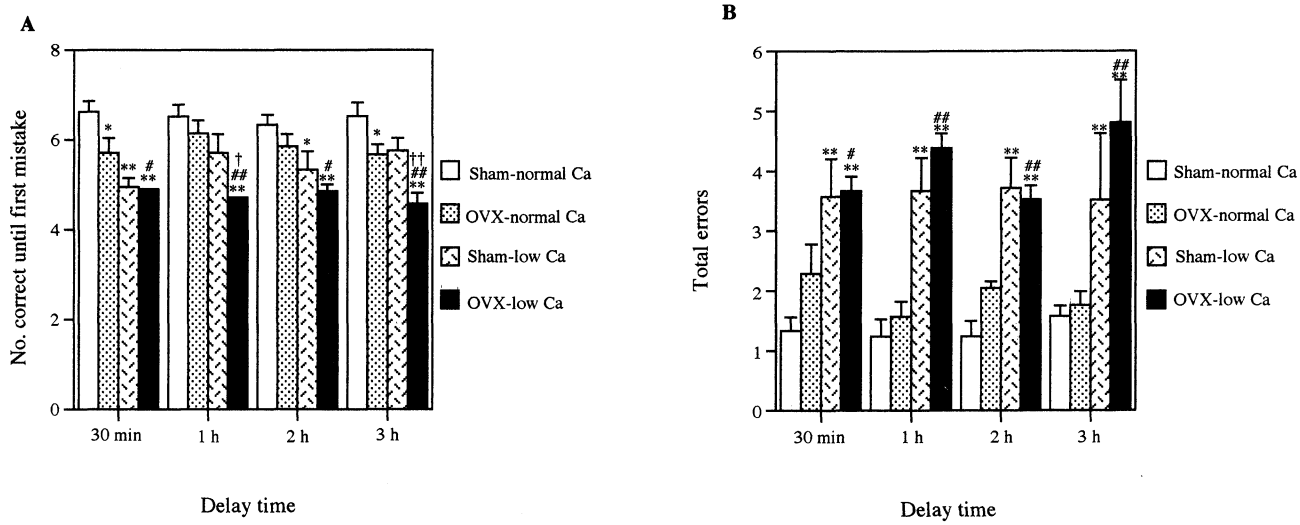

Fig. 2. Effects on performance of introducing each time delay (30 min-3 h) between the fourth and fifth choices. Values are the average number of correct choices until the first mistake±S.E.M. (A) and the average total number of incorrect choices±S.E.M. (B) for seven rats per group. Each time-delay period is the mean of three consecutive trials. Open bars: normal control diet plus sham-ovariectomy (Sham-normal Ca); dotted bars: normal control diet plus ovariectomy (OVX-normal Ca); hatched bars: low-calcium diet plus sham-ovariectomy (Sham-low Ca); solid bars: low-calcium diet plus ovariectomy. (OVX-low Ca) *$P<0.05$ and **$P<0.01$ vs. Sham-normal Ca group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. OVX-normal Ca group; $^{\dagger}P<0.05$ and $^{\dagger\dagger}P<0.01$ vs. Sham-low Ca group.

terion are shown in Fig. 3A and the number correct until the first mistake and the total number of errors are shown in Fig. 3B. The number correct until first mistake and the total errors in Fig. 3B were calculated as the average for the five consecutive trials until the criterion was fulfilled. As for the trial to criterion (Fig. 3A), two-factorial ANOVA revealed significant differences between the normal- and low-calcium diets (F (1,48)=14.35, P<0.01) and among the hormonal treatments (F (3,48)=9.06, P<0.01) but the interaction between the calcium diet and hormonal treatment was not significant (F (3,48)=1.66). In short, low-calcium diet groups had higher scores than the normal-calcium groups (i.e. more trials to criterion). Post hoc tests showed a significant difference between the OVX-normal-Ca and E2 (P<0.01), OVX-normal-Ca and E2+P4 (P<0.01), P4 and E2+P4 (P<0.05), E2+P4 and LE2+LP4 (P<0.01), OVX-low-Ca and LE2 (P<0.01), and LE2 and LE2+LP4 (P <0.01) groups. Although statistical significance was not detected, the number of trials until criterion of the OVX-normal-Ca group tended to be lower than that of OVX-low-Ca group (P=0.07). Also, the trials to criterion of the E2 and E2+P4 groups were lower than those of the OVX-normal-Ca and P4 groups, i.e. the OVX-normal-Ca and P4 groups satisfied the criterion in about 23 and 20 trials, respectively, while the E2 and E2+P4 groups satisfied it in about 14 and 13 trials, respectively. Furthermore, the number of trials to criterion was substantially higher in the OVX-low-Ca group than in the 17β-estradiol-treated group, i.e. the LE2 group satisfied the criterion in about 16 trials, while the OVX-low-Ca group did not satisfy the criterion until about 28 trials. Additionally, the LP4 and LE2 +LP4 groups did not satisfy the criterion until 25 trials. However, there were no significant differences between the OVX-low-Ca and LP4, and LP4 and LE2+LP4 groups.

As for the number of correct choices Fig. 3B, two-factorial ANOVA showed significant differences between normal- and low-Ca groups (F(1,48)=6.99, P<0.05), though the effect of hormonal treatment and the calcium diet × hormonal treatment interaction were not significant. However, post hoc tests showed that there were tendencies

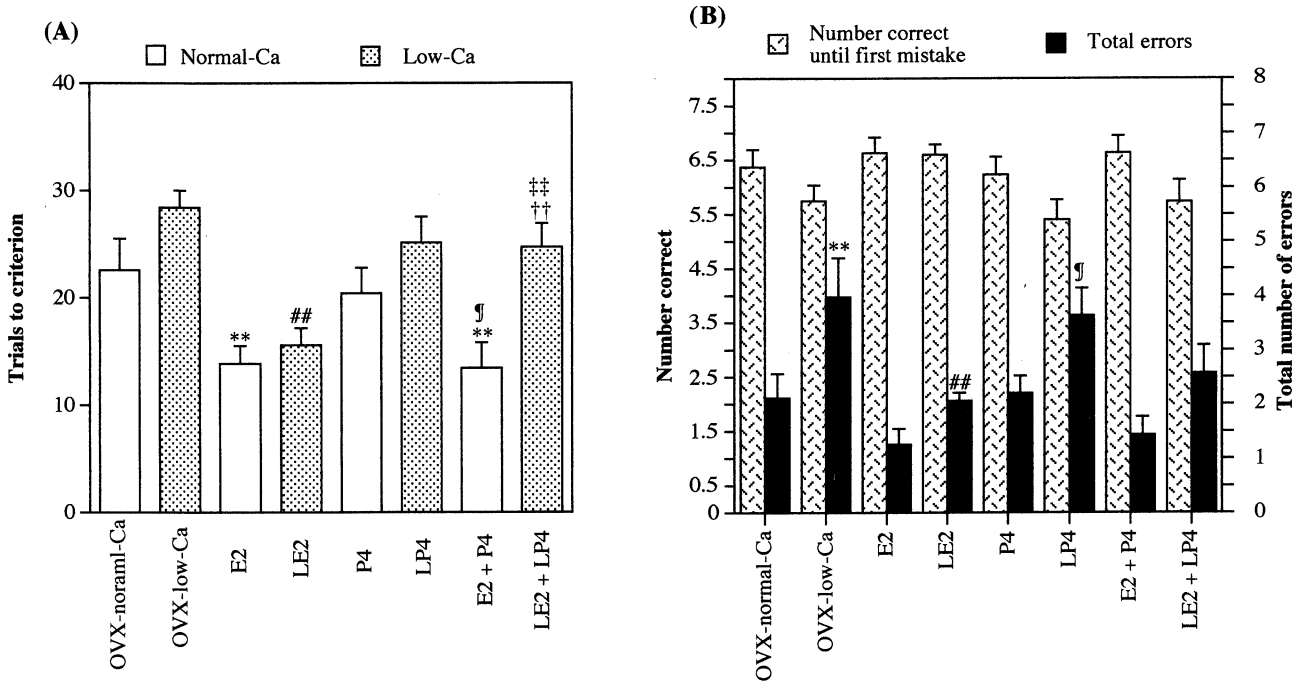

Fig. 3. The number of trials until the criterion was reached (A) and choice accuracy (B) in the regular trial in rats with normal- and low-calcium diets. The choice accuracy was evaluated by "number correct until first mistake" and "total number of errors." The number correct until first mistake and the total number of errors were calculated as averages for the five consecutive trials until the performance criterion was reached. Results are expressed as the mean±S.E. M. (n=7 per group). Comparisons of changes among different treatment groups were made using two-factorial ANOVA with post hoc tests. $^{**}P$<0.05 vs. OVX-normal-Ca group; $^{\#\#}P$<0.01 vs. OVX-low-Ca group; $^{¶}P$<0.05 vs. P4 group; $^{\dagger\dagger}P$<0.01 vs. E2+P4 group; $^{\ddagger\ddagger}P$<0.05 vs. LE2 group.

toward to higher scores in the E2+P4 group versus the LE2+LP4 group (P=0.052) and in the LE2 group versus the LE2+LP4 group (P=0.06) in the number of correct choices. Similarly, as for the total errors, two-factorial ANOVA revealed significant differences for the calcium diets (F (1,48)=17.33, P<0.01) and the hormonal treatments (F (3,48)=4.67, P<0.01), while the calcium diet × hormonal treatment interaction was not significant. Post hoc tests showed significant differences between the OVX-normal-Ca and OVX-low-Ca (P<0.01), OVX-low-Ca and LE2 (P <0.01), and P4 and LP4 (P<0.05) groups. The analysis also showed a tendency towards higher scores in the LE2+LP4 than in the E2+P4 group (P=0.07) in the total errors, although there were no significant differences between the OVX-low-Ca and LE2+LP4, and LP4 and LE2+LP4 groups. In short, treatment with 17β-estradiol promoted task acquisition, while treatment with progesterone did not alter the facilitative effect of estradiol on task acquisition in rats fed a normal-calcium diet. Although the treatment with progesterone alone did not affect the number of trials to criterion, the number correct until first mistake, or the total errors of the OVX-low-Ca group, progesterone treatment abolished the effect of estradiol on the performance in animals maintained on the low-calcium diet.

Fig. 4 shows the number of correct choices until the first mistake (Fig. 4A) and the total number of errors in a block (Fig. 4B) for rats consuming normal- and low-calcium diets. Each trial block consisted of six consecutive trials. As for the number of correct choices until the first mistake, two-factorial ANOVA revealed significant differences between normal- and low-calcium diets at blocks 3 (F (1,48)=25.25, P<0.01), 4 (F (1,48)=42.3, P<0.01), and 5 (F (1,48)=40.51, P<0.01), and among the hormonal treatments groups at blocks 2 (F(3,48)=12.43), 3 (F(3,48)=9.60, P<0.01), 4 (F(3,48)=2.53, P<0.01), and 5 (F(3,48)=12.8, P <0.01). There was a significant interaction of calcium diet × treatment in block 5 only (F(3,48)=3.47, P<0.05). Although not statistically significant, a calcium diet × hor-

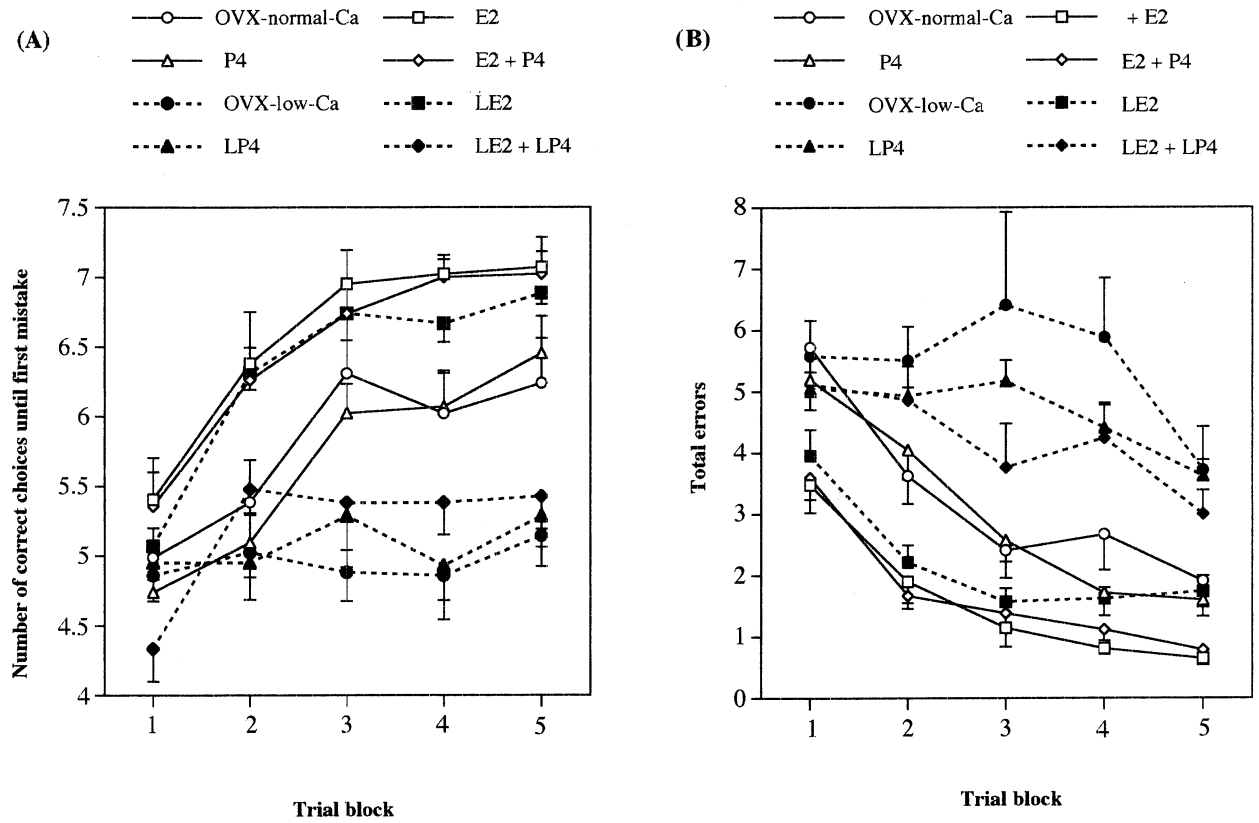

Fig. 4. Changes in the number of correct choices until the first mistake (A) and total number of incorrect choices (B) for maze performance of each hormonal treatment and diet group in rats. The values are the average number of correct choices until the first mistake ± S.E.M. (A) and the average total number of error choices ± S.E.M. (B) for seven rats per group. A block is the mean of six consecutive trials.

monal treatment interaction trend was observed for blocks 3 ($F(3,48)=2.37$, $P=0.08$) and 4 ($F(3,48)=2.53$, $P=0.07$). The results of statistical analysis indicated that the change in the number of correct choices until the first mistake differed for each treatment group during the regular trials (i.e. each treatment group differed in its ability to resolve the radial maze task). Furthermore, we compared each treatment group by post hoc tests on each trial block, and there were significant differences between the following groups: OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.05$), P4 and E2+P4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.05$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.05$) in block 2; OVX-normal-Ca and OVX-low-Ca ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 3; OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.01$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and E2+P4 ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 4; and OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 5. There were no significant differences between the OVX-normal-Ca and P4, E2 and E2+P4, OVX-low-Ca and LP4, OVX-low-Ca and LE2+LP4, and LP4 and LE2+LP4 groups in any trial block.

As for the data shown in Fig. 4B, two-factorial ANOVA revealed significantly different effects of the calcium diets in blocks 2 (F (1,48)=26.7, $P<0.01$), 3 (F (1,48)=18.42, $P<0.01$), 4 (F (1,48)=41.16, $P<0.01$), and 5 (F (1,48)=47.71, $P<0.01$) and of the hormonal treatments in blocks 1 (F (3,48)=4.20, $P<0.05$), 2 (F (3,48)=15.26, $P<0.01$), 3 (F (3,48)=26.23, $P<0.01$), 4 (F (3,48)=10.82, $P<0.01$), and 5 (F (3,48)=8.13, $P<0.01$). There was also a significant interaction of calcium diet×treatment at block 2 (F (3,48)=4.34, $P<0.01$). Although not statistically significant, a calcium diet×hormonal treatment trend was apparent for block 3 (F (3,48)=1.80, $P=0.10$) and 4 (F (3,48)=2.14, $P=0.11$). Furthermore, we also compared each treatment group by post hoc tests in each trial group, and found significant differences between the following groups: OVX-normal-Ca and E2 ($P<0.01$) and OVX-normal-Ca and E2+P4 ($P<0.05$) in block 1; OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.01$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and E2+P4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 2; OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.05$), E2+P4 and LE2+LP4 ($P<0.05$), OVX-low-Ca and LE2 ($P<0.01$), OVX-low-Ca and LE2+LP4 ($P<0.05$), and LE2 and LE2+LP4 ($P=0.05$) in block 3; OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), OVX-low-Ca and LE2+LP4 ($P<0.05$), and LE2 and LE2+LP4 ($P<0.01$) in block 4; and OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.05$), E2 and LE2 ($P<0.05$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$, and LE2 and LE2+LP4($P<0.05$) in block 5.

3) Effect of steroid hormones on $(Na^+ + K^+)$-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task in gonadectomized mice[3].

To examine the possible roles and mechanism of action of steroid hormones against amnesia induced by ouabain, an inhibitor of (Na^+, K^+)-ATPase, gonadectomized male mice were administrated ouabain (0.1 μg per mouse) intracisternally (i.cist.), and the learning and memory abilities of the mice were assessed by a step-through passive avoidance task. Subcutaneous (s.c.) administration of 17β-estradiol (βE2; 10 μg kg^{-1}) or testosterone (TES; 1 mg kg^{-1}) improved the memory impairment induced by ouabain, while administration of dihydrotestosterone (1 mg kg^{-1}) or corticosterone (COR) (1 mg kg^{-1}) did not. Treatment with the estradiol receptor antagonists, tamoxifen (TAM) (10 mg kg^{-1}; s.c. or 0.1 μg; i.cist.) and 4-hydroxytamoxifen (10 mg kg^{-1}; s.c.), or the androgen receptor antagonist, cyproterone (10 mg kg^{-1}; s.c. or 1 μg; i.cist.), did not influence the protective effect of βE2 or TES on ouabain-induced amnesia. Moreover, we studied the effects of several free radical scavengers-17α-estradiol (10 μg kg^{-1}; s.c.), α-tocopherol (VE: 200 mg kg^{-1}; per os (p.o.), ascorbic acid (VC: 200 mg kg^{-1}; p.o.), or VE+VC (200 mg kg^{-1} each; p.o.) on ouabain-induced amnesia, and compared those effects with that of βE2. The

administration of free radical scavengers had no significant effect on memory impairment. These results indicate that βE2 and TES ameliorate the amnesia induced by inhibition of (Na^+, K^+)-ATPase activity, and that the protective effect of βE2 is caused by a non-genomic, rather than a genomic effect or a radical scavenging action. Additionally, the ameliorative effect of TES does not appear to involve free radical scavenging, but its aromatization to estrogen could contribute to the non-genomic action of βE2.

Above abstract was derived mainly from following experimental results[2].

Effects of steroid hormones on ouabain-induced amnesic and ouabain-untreated mice in the step-through passive avoidance test was determined. The effects of steroid hormones on ouabain-induced amnesia (Fig. 5A) differed significantly among the treated groups (F (6,59) =2.67; P<0.05). Post hoc test showed that pretreatment with βE2 (10 μg kg^{-1}) significantly inhibited (P<0.01) the ouabain-induced amnesic effect on latency time for the step through latency trial (STL). TES, a testicular steroid hormone, did not have a significant effect on ouabain-induced amnesia at a dose of 0.1 mg kg^{-1}, but showed a significant protective effect at 1 mg kg^{-1} (P<0.05). DHT, a non-aromatizable androgen, at doses of 0.1 and 1 mg kg^{-1} did not significantly inhibit ouabain-induced impairment of learning and memory. The administration of COR (0.1 or 1 mg kg^{-1}), an endogenous adrenocortical steroid hormone in rats and mice, did not have a significant effect on ouabain-induced amnesia. Additionally, the administration of βE2 (10 μg kg^{-1}) and TES (1 mg kg^{-1}) did not have a significant effect in sham-operated or gonadectomized (GOX) -mice that were not treated with ouabain (Fig. 5B).

As shown in Fig. 5, TES at a dose of 1 mg kg^{-1} protected against ouabain-induced amnesia in the step-through passive avoidance task. Since the hypothalamus is capable of aromatizing a small proportion of testosterone to estradiol[40] or androstenedione to oestrone[41], the protective effect of TES could result from its aromitization to an estrogen. This possibility was further suggested by the observation that dihydrotestosterone (DHT), a non-aromatizable androgen, did not protect against ouabain-induced amnesia (Fig. 5). To further address this possibility, we studied whether the protective effect of TES against ouabain-

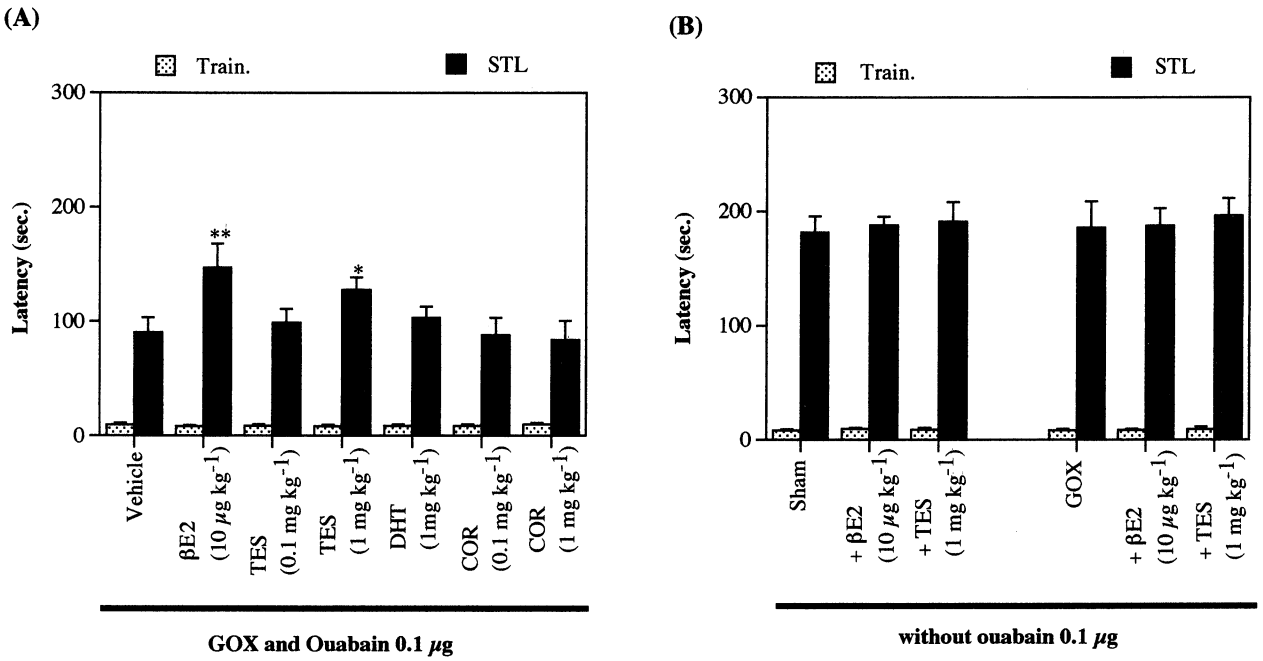

Fig. 5. Effects of steroid hormones on ouabain-induced amnesia (A) and on untreated Sham and GOX-mice in the step-through passive avoidance task. Steroid pretreatment was given 5 days before the Train. The mice were gonadectomized and injected intracisternally with 0.1 μg ouabain (A), or treated with same volume of vehicle without ouabain (B). Values are mean±S.E.M. for 5-10 animals per group. *P<0.05; **P<0.01 vs. STL in the vehicle group (by post hoc test). βE2=17 β-estradiol, TES: testosterone, DHT: dihydrotestosterone, COR: corticosterone.

induced amnesia was mediated by andorogen receptors.

We studied the influence of TAM (i.cist.) and 4-hydroxytamoxifen (HYT; s.c.) on the recovery from ouabain-induced memory impairment by βE2 (Fig. 6). Although ANOVA revealed a significant difference between groups (F (5,52)=2.44; P<0.05), the following post hoc test showed that the treatment with TAM at 0.1 μg (i.cist.) did not affect the βE2-induced improvement of latency time on the STL. In addition, HYT is a metabolite of tamoxifen, and is a more potent estrogen antagonist than TAM[42]. However, this more potent antagonist at a dose of 10 mg kg^{-1} (s.c.) did not inhibit the recovery from ouabain-induced amnesia caused by βE2.

Fig.7 shows the influence of cyproterone, an antiandrogen, on the protective effect of TES against ouabain-induced impairment of learning and memory in the step-through passive avoidance task. Cyproterone was given s.c. at a dose of 10 mg kg^{-1}, which was reported to antagonize the action of 1 mg kg^{-1} TES[43], and the i.cist. doses used were 0.1 and 1 μg per mouse. The s.c. or i.cist. administration of cyproterone alone did not affect ouabain-induced amnesia in the step-through passive avoidance task, and cyproterone administered s.c. or i.cist. in combination with TES did not significantly modify the protective action of TES against ouabain-induced amnesia. Cyproterone at a dose of 0.1 μg (i.cist.) also had no effect on ouabain-induced amnesia or the protective effect of 1 mg kg^{-1} TES.

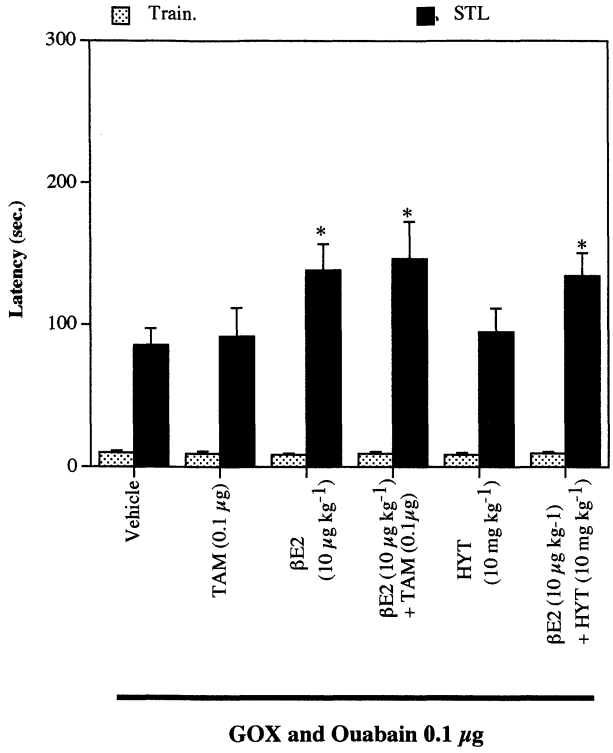

Fig. 6. Influence of TAM and HYT on the protective effect of βE2 against ouabain-induced amnesia in the step-through passive avoidance task. All mice were gonadectomized, injected with 0.1 μg ouabain i.cist. and given either vehicle, βE2 (10 μgkg^{-1}; s.c.), TAM (0.1 μg; i.cist.), HYT (10 mg kg^{-1}; s.c.), βE2 +TAM, or βE2+HYT. Neither TAM nor HYT influenced the latency time on the retention trial of βE2-treated mice. Values are mean±S.E.M. for 8-12 animals per group. *P<0.05 vs. STL in the vehicle group (by post hoc test). βE2=17 β-estradiol, TAM: tamoxifen, HYT: 4-hydroxytamoxifen.

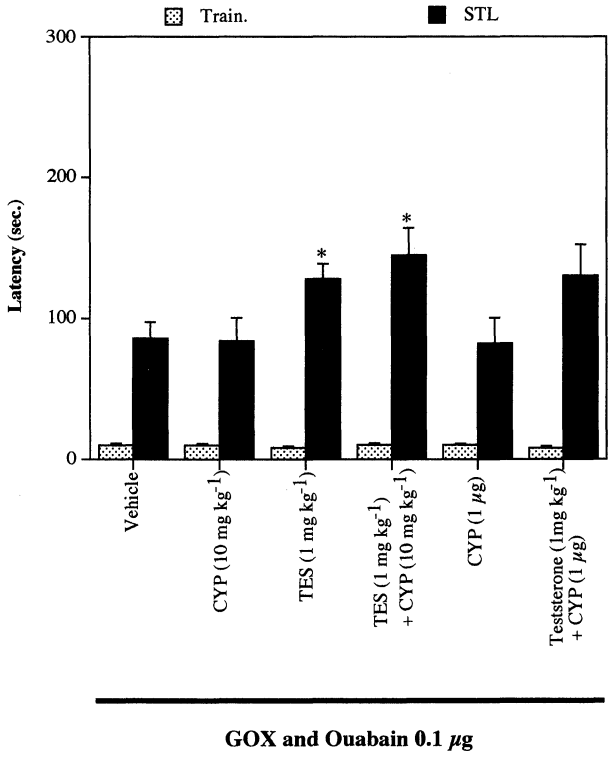

Fig. 7. Influence of cyproterone treatment on the protective effect of TES against ouabain-induced impairment of learning and memory in the step-through passive avoidance task. All mice were gonadectomized and injected with 0.1 μg ouabain i.cist. and then given TES (1 mg kg^{-1}; s.c.), CYP (10 mg kg^{-1}; s.c. or 1 μg; i.cist.) or TES+CYP (either s.c. or i.cist.). Both doses of CYP failed to affect the recovery from ouabain-induced amnesia by TES. Values are mean ±S.E.M. for 8-10 animals per group. *P<0.05 vs. STL in the vehicle group (by post hoc test). TES: testosterone, CYP: cyproterone.

As mentioned above, the protective effect of TES suggests that estradiol, which can be generated by the aromatization of TES, could affect ouabain-induced amnesia via estradiol receptors. We, therefore, examined the effect of TAM on the protective action of TES against ouabain-induced amnesia in the step-through passive avoidance task. As shown in Fig. 8, 0.1 μg TAM (i.cist.) did not influence the protective action of TES (1 mg kg^{-1}).

Conclusive remarks

As mentioned above, it seems that estradiol alone has a beneficial effect on the learning and memory, or mood formation. However, when estradiol was used together with progesterone, negative effects appear. Thus, neuroactive steroids may play important distinct roles in the regulation of the learning and memory, or the mood formation probably through their non-genomic or indirect action rather than a genomic action on their receptors, but the mechanisms behind their effects are not clear and must be wait for the future research.

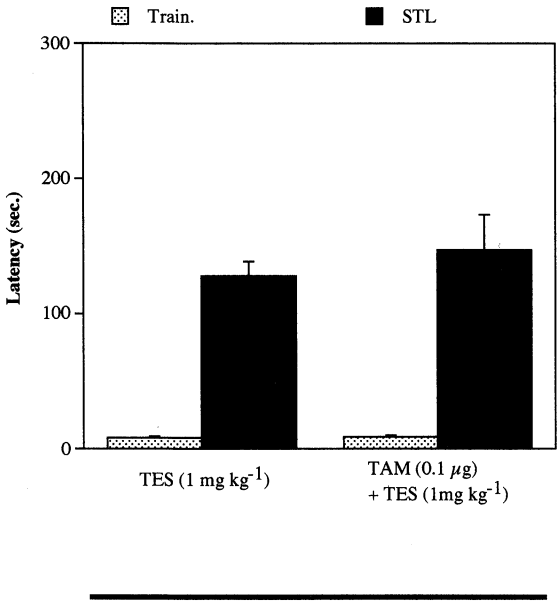

Fig. 8. The effect of TAM on TES-mediated protection against ouabain-induced amnesia in the step-through passive avoidance task. All mice were gonadectomized and injected with 0.1 μg ouabain i.cist., and given TES (1 mg kg^{-1}; s.c.) or TES+TAM (0.1 μg; i.cist.). TAM did not have a significant effect on the recovery from ouabain-induced amnesia by TES. Values are mean±S.E.M. for 8-10 animals per each group. TES: testosterone, TAM: tamoxifen.

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