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A Monoclonal Antibody to Oestradiol Potentiates the Stimulation of the Specific Activity of the Brain Type Creatine Kinase by Oestrogen *in vivo* and *in vitro*

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We described previously the *in vivo* immunoneutralization effects of a high affinity anti-oestradiol antibody clone 15 in blocking ovulation and synaptic remodeling in cycling female rats. In the present study we report the enhancing effects of this antibody. Treatment of ovariectomized female rats or female derived skeletal cell cultures *in vitro* with anti-E_2 15 plus oestrogen (E_2) potentiated the specific activity of the brain type creatine kinase (CK) response to E_2 in the rat tissues or skeletal cells. The enhancing CK response of anti E_2 15 plus E_2 was time- and dose-dependent in the uterus, thymus, epiphysis and diaphysis of ovariectomized female rats. In the pituitary, on the other hand, anti-E_2 15 blocked the stimulatory CK response to E_2. Two other high affinity anti-E_2 antibodies, clones $8D_9$ and $11B_6$, had no effect in augmenting the response of CK to E_2 in rat tissues. Moreover, the enhancing CK response in rat tissues was specific to anti-E_2 15 plus E_2 since the intact anti-E_2 in the presence of other oestrogen mimetics, such as oestriol or stilbestrol or tamoxifen did not potentiate the CK response in rat tissues. In this model system the Fab′ monomer of anti-E_2 15 abolished the CK response to E_2 in rat tissues and not to anti-E_2 15 plus E_2 whereas tamoxifen completely blocked the CK response to anti E_2 plus E_2. Anti E_2 15 may therefore serve as a specific carrier in delivering E_2 to oestrogen sensitive rat tissues or cells containing functional oestrogen receptors and thereby increasing the magnitude of E_2 effects *in vivo* and *in vitro*. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Using hybridoma technology [1] we have produced and characterized several cell lines that secrete monoclonal anti-oestradiol (E_2) antibodies, with high affinity and specificity to oestradiol [2]. These hybridomas were derived from the fusion of mouse myeloma cells with spleen cells of different strains of mice immunized with oestradiol-6-carboxymethyl oxime conjugated to bovine serum albumin (BSA) or thyroglobulin. In pilot experiments we used one of the highly specific clones, anti-E_2 15, to block the effect of oestrogen during the rat oestrus cycle. Treatment of female rats on the day of dioestrus with anti-E_2 15 resulted in the complete blockage of ovulation [3]. Anti-E_2 15 had no effect when it was injected on the day of proestrus. Interestingly, another high affinity anti-E_2 antibody (clone $8D_9$) had no effect in blocking ovulation in rats. Moreover, we could demonstrate that anti-E_2 15 selectively inhibited oestrogen induced synaptic plasticity only in the periventricular zone of reaction, during the oestrus cycle [3]. In additional experiments we showed that six months treatment of mice induced with experimental lupus erythematosus (SLE) with anti-E_2 15 had beneficial effects on the clinical manifestations of the disease [4].

In this paper both D. S. and Y. A.-Z. can be considered first authors.

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Due to our interest in using antibodies as probes for hormone action, in the present paper we examined the effects of three antibodies to oestradiol, clones 15, $8D_9$ and $11B_6$, respectively, on the specific activity of creatine kinase (CK) induced by oestrogen *in vivo* in rat tissues and *in vitro* in skeletal derived cells responsive to oestrogen. CK was chosen as a response marker since we have previously shown that CK can be used as a response marker for oestrogenic activity *in vivo* in rat animal models [5] and *in vitro* in skeletal derived cells [6]. Moreover, we have reported that CK is related to changes in cell division [7–10] and CK can be used as a marker for the interaction of E_2 with the functional oestrogen receptor (ER) [11].

In the present paper we report that only clone 15 and neither clone $11B_6$ nor clone $8D_9$ can enhance *in vivo* the CK specific activity to oestrogen in a variety of rat organs containing functional ER. In addition, the proteolytic fragment, the Fab' monomer of anti-E_2 15, behaves like an antagonist *in vivo*, suggesting that the intact antibody is necessary for the potentiation of CK response to E_2. This enhancing CK response to E_2 by anti E_2 15 is not limited to *in vivo* since the upregulation of CK specific activity response to oestrogen is also observed *in vitro* when skeletal cells are treated similarly.

MATERIALS AND METHODS

Generation of monoclonal antibodies to 17β-oestradiol (E_2)

Three different strains of mice (C57B6, CB6F1 and CD2) respectively, were immunized with oestradiol-6-carboxymethyl oxime bovine serum albumin or with oestradiol-6-carboxymethyloxime thyroglobulin conjugate. The spleen cells of the immunized mice were fused with mouse myeloma line P3/NSI-Ag or NSO, using the technique of Köhler and Milstein [1], with the modifications developed in our laboratory [2]. Hybridomas secreting antibodies to oestradiol were identified by radioimmunoassay or by time resolved fluorescence. Clones 15, $8D_9$ and $11B_6$ were characterized as IgG_{2b}, IgG_{2a} and IgG_{2b} (Table 1), respectively. Cross-reactivity of each monoclonal with related compounds was determined by a competitive binding immunoassay (Table 1). The affinity (K_D) of all monoclonals for estradiol was in the range of 1 nM. Hybridoma cells were grown as ascites in pristane primed male mice. Purification of antibodies from ascitic fluid was carried out on Sepharose Protein A, as described previously [12]. The Fab' monomer of clone 15 was prepared by immobilized papain digestion of clone 15, followed by affinity chromatography on Sepharose Protein A, as described previously [7]. Mouse IgG_{2b} was prepared by binding of normal mouse serum on Sepharose Protein A, followed by elution with 0.1 M citrate buffer, at pH 3. The heavy chain class of the eluted fractions was determined using the Ochterlony double immunodiffusion assay and the Mouse Antibody Isotyping Kit.

Animals

Wistar derived female rats from the Biological Regulation Departmental colony were used at the age of 25 days. Gonadectomy of female rats was carried out at the age of 25 days, and the various treatments were carried out 2 weeks after surgery. The colony was housed in air-conditioned quarters with light

Table 1. Properties of hybridoma cell lines secreting antibodies to oestradiol (E_2)

Compound	Cross-reaction (%) Clone #		
	15	$8D_9$	$11B_6$
Oestradiol	100	100	100
Oestriol	60	<0.1	100
Estrone	<0.1	<0.1	<0.1
Epi-oestriol	100	100	100
Cortisol	<0.01	<0.01	<0.01
Testosterone	<0.01	<0.01	<0.01
Progesterone	<0.01	<0.01	<0.01
Tamoxifen	<0.001	<0.001	<0.001
Stilbestrol	<0.001	<0.001	<0.001
Heavy chain class	IgG_{2b}	IgG_{2a}	IgG_{2b}
Affinity (K_D)	1 nM	1 nM	1 nM
Strain of immunized mice	Balbc	CB_6F_1	CD_2
Immunogen	E2-6..BSA[a]	E2-6..BSA[a]	E2-6..thyroglobulin[b]

[a]E_2-6..BSA: oestradiol-6-carboxymethyl bovine serum albumin.
[b]E_2-6..thyroglobulin: oestradiol-6-carboxymethyl.

from 05.00 to 19.00 h and had free access to Purina Laboratory Chow and water.

Reagents

All reagents used were of analytical grade. Biochemicals, steroids, reagents, mouse serum and mouse antibody Isotyping Kit and CK assay kit were obtained from Sigma Chemical (St. Louis, MO). Sepharose-Protein A was from Pharmacia (Uppsala). Immobilized-papain was purchased from Pierce (Rockford, IL).

Hormonal treatment

The changes in CK activity in the epiphyseal cartilage, diaphyseal bone, uterus, thymus and pituitary induced by the short-term treatment of oestradiol (E_2), monoclonal anti-oestradiol antibody, mouse IgG_{2b}, oestrogenic mimetics, and combinations of oestrogenic compounds with monoclonal anti-oestradiol antibodies were studied in gonadectomized female rats, using 5 animals in a group, and the doses indicated for each experiment. Injection of the animals with 0.05% ethanol in phosphate buffered saline (PBS) served as control for the steroid group whereas injection of mouse IgG_{2b} alone served as control for the groups injected with the various antibodies. A dose of 5 μg of E_2 per rat was used since this dose is optimal for bone tissue. The animals were killed by decapitation, 24 h after the i.p. injection (unless otherwise stated). The various organs were removed, stored at −20°C until processed for CK activity.

Enzyme extraction and assay

Rat organs from gonadectomized rats were homogenized using a Polytron model PTA 105 (Kinematica, Littan) in 1 ml homogenization buffer [8, 10] consisting of 50 mM Tris HCl (pH 6.8), 5 mM magnesium acetate, 2.5 mM dithiothreitol, 0.4 mM EDTA, and 250 mM sucrose. Cytosolic extracts were obtained by centrifugation of homogenates at 14,000 × g for 5 min at 4°C in an Eppendorf microcentrifuge. Creatine kinase (CK) specific activity was measured in a Uvicon automatic recording spectrophotometer (Kontron, Switzerland) at 340 nm, using a coupled assay, in 0.7 ml incubation mixture obtained from Sigma CK assay kit (Sigma, St. Louis, MO). A unit of enzyme activity was defined as the amount yielding 1 μmol adenosine triphosphate/min at 30°C, and specific activity as units per milligram protein. Protein was determined by Coomassie blue binding dye [13] using BSA as the standard.

Cell cultures

19–21-day-old rat embryo calvaria cell cultures were prepared from female embryos and maintained as previously described [8], in BGJb medium supplemented with 10% fetal calf serum. ROS 17/12.8 cells were cultured as described previously [14]. Cell cultures were treated with various reagents after 5 days when they reached confluence. CK activity in cell extracts was determined after 24 h.

Statistical analysis

The significance of differences between experimental and control means was evaluated by unpaired two-tailed student's t-test and by analysis of variance (ANOVA).

RESULTS

Characterization of anti-oestradiol antibodies

All the three antibodies were able to bind oestradiol and epi-oestriol with high affinity (K_D 1 nM), showed minimal cross-reaction with oestrone (<0.1%) and almost no significant cross-reaction (<0.001) with other steroids (progesterone, cortisol, testosterone) and with oestrogenic mimetics (stilbestrol, tamoxifen). However, notable differences were noted with respect to oestriol. Clones 15 and $11B_6$ recognized oestriol well (range of 60 to 100%) and clone $8D_9$ showed minimal cross-reaction (0.1%) with oestriol (see Table 1).

Dose dependent stimulation of the specific activity of CK by oestrogen plus anti-oestradiol 15 in vivo

Treatment of immature ovariectomized rats with various amounts of anti-E_2 15 in the presence of 5 μg oestrogen for 24 h led to a dose dependent potentiation of CK response to oestrogen in the epiphysis, diaphysis, thymus and uterus. The enhancing effect of anti-E_2 was effective when administered at a dose of 12.5 μg antibody/rat and reached a maximum at a dose of 25 μg antibody/rat (Table 2). On the other hand, administration of E_2 (5 μg) plus mouse IgG_{2b} (25 μg) or anti-E_2 8D9 (25 μg) did not enhance the CK response to oestrogen in these tissues. Moreover, the potent oestrogen mimetic stilbestrol (5 μg) caused a significant increase in CK activity in all these tissues. However, the combination of stilbestrol plus anti-E_2 15 did not enhance the CK response to stilbestrol. In addition, anti E_2 15 alone did not stimulate CK activity (Table 2).

Time-dependent stimulation of the specific activity of CK by oestrogen plus anti-oestradiol 15 in vivo

Ovariectomized female rats were injected at various times with oestrogen plus anti-E_2 15 or with anti-E_2 15 alone or E_2 alone or with mouse IgG plus E_2. E_2 by itself stimulated CK, but only the combination of anti-E_2 15 and E_2 led to a potentiation of CK response to oestrogen as early as 3 h after treatment in the diaphysis, epiphysis and uterus (Table 3). A plateau was reached six hours after injection of anti-E_2 15 and E_2 (Table 3) and remained elevated 24 h after

Table 2. Dose-dependent stimulation of specific activities of CK by oestrogen plus various amounts of anti-oestradiol 15 in vivo

Treatment	CK specific activity (E/C) Organ			
	Uterus	Diaphysis	Epiphysis	Thymus
Control	1.00 ± 0.1	1.00 ± 0.22	1.00 ± 0.28	1.00 ± 0.13
E_2 (5 μg)	1.72 ± 0.05^{b}	2.05 ± 0.16^{b}	1.89 ± 0.09^{a}	1.45 ± 0.15^{b}
IgG (25 μg)	1.07 ± 0.08	0.96 ± 0.13	1.17 ± 0.10	0.96 ± 0.11
Ab 15				
12.5 μg	0.96 ± 0.15	0.99 ± 0.21	1.26 ± 0.2	0.89 ± 0.10
25 μg	1.01 ± 0.125	1.41 ± 0.41	1.35 ± 0.33	1.03 ± 0.11
50 μg	0.84 ± 0.36	1.23 ± 0.25	1.36 ± 0.17	1.10 ± 0.17
Ab 15 + E_2(5 μg)				
12.5 μg	1.84 ± 0.09^{b}	2.27 ± 0.08^{b}	1.78 ± 0.10^{a}	1.49 ± 0.16^{b}
25 μg	$2.38 \pm 0.07^{c,e}$	$3.26 \pm 0.05^{c,e}$	$2.19 \pm 0.06^{a,d}$	$1.70 \pm 0.11^{b,d}$
50 μg	$2.42 \pm 0.16^{b,d}$	$2.99 \pm 0.12^{c,d}$	$2.64 \pm 0.02^{b,e}$	1.28 ± 0.2
IgG (25 μg) + E_2(5 μg)	1.69 ± 0.16^{b}	2.18 ± 0.14^{c}	1.93 ± 0.24^{b}	1.68 ± 0.20^{a}
Ab 8D9 (25 μg) + E_2	1.48 ± 0.03^{a}	2.13 ± 0.10^{c}	2.11 ± 0.22^{b}	1.46 ± 0.13^{a}
DES (5 μg)	2.06 ± 0.08^{b}	2.57 ± 0.26^{c}	2.76 ± 0.14^{c}	1.98 ± 0.11^{b}
DES + Ab 15 (25 μg)	1.86 ± 0.20^{b}	2.70 ± 0.19^{c}	2.31 ± 0.24^{b}	1.74 ± 0.28^{a}

Twenty-five day old female rats were used 2 weeks after ovariectomy, injected with various amounts of Ab 15 plus E_2 (5 μg/rat), mouse IgG_{2b} (25 μg/rat), mouse IgG_{2b} (25 μg/rat) plus E_2 (5 μg/rat) or E_2 (5 μg/rat) and the various organs assayed for specific CK activity as described previously [2, 3]. Results are expressed as mean ± S.E.M. for $n = 5$–10.

[a] $P \leq 0.05$.

[b] $P \leq 0.01$.

[c] $P \leq 0.001$ for the statistical significance compared to the untreated control group.

[d] $P \leq 0.05$.

[e] $P \leq 0.01$ for the statistical difference between Ab15 + E_2 vs E_2 at each point.

Table 3. Time-dependent stimulation of the specific activity of CK by oestrogen plus anti-oestradiol 15 in vivo

Treatment	CK specific activity (E/C) Organ		
	Uterus	Diaphysis	Epiphysis
Control	1.00 ± 0.06	1.00 ± 0.06	1.00 ± 0.13
E2 (5 μg)			
3 h	1.39 ± 0.08^{a}	1.59 ± 0.26^{a}	1.47 ± 0.11^{a}
6 h	1.72 ± 0.09^{a}	1.89 ± 0.22^{b}	1.75 ± 0.07^{a}
24 h	1.62 ± 0.15^{a}	2.11 ± 0.09^{b}	1.72 ± 0.14^{a}
Ab 15 (25 μg)			
3 h	1.31 ± 0.29	0.81 ± 0.22	1.03 ± 0.08
6 h	1.16 ± 0.10	1.01 ± 0.20	1.09 ± 0.06
24 h	1.06 ± 0.26	1.02 ± 0.19	1.06 ± 0.16
Ab 15 (25 μg) + E_2 (5 μg)			
3 h	1.32 ± 0.13^{a}	1.50 ± 0.03^{a}	1.65 ± 0.15^{a}
6 h	$2.07 \pm 0.20^{b,d}$	$3.50 \pm 0.08^{c,d}$	$2.34 \pm 0.05^{b,d}$
24 h	$2.25 \pm 0.16^{b,d}$	$3.74 \pm 0.13^{c,e}$	$2.32 \pm 0.12^{b,d}$

Immature female rats were used 2 weeks after ovariectomy, and injected for the times indicated with [Ab15 (25 μg/rat) plus E_2 (5 μg/rat)], mouse IgG_{2b} (25 μg/rat) plus E_2 (5 μg/rat), Ab15 (25 μg/rat) or E_2 (5 μg/rat). The various organs were assayed for CK activity at the indicated times. The results are expressed as means ± S.E.M., for $n = 5$–10.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$ for the statistical significance compared to the untreated control.

[d] $P < 0.05$.

[e] $P < 0.01$ For the statistical difference between Ab15 + E_2 vs E_2 at each point.

treatment. Anti-$E_2$15 alone did not stimulate CK specific activity (Table 3). Mouse IgG plus E_2 gave similar results as E_2 alone (data not shown).

Structural diversity of the anti-oestradiol antibodies in enhancing the CK response to E_2 in vivo

Ovariectomized female rats were treated for 24 h with oestradiol in the presence of either mouse IgG_{2b}, anti-E_2 15, the Fab′ of anti-E_2 15, anti-E_2 $8D_9$, or anti-E_2 $11B_6$. Only the combination of anti-E_2 15 plus oestradiol enhanced the CK response to oestrogen in the diaphysis, epiphysis and uterus (Fig. 1). The other two anti-oestradiol antibody clones $8D_9$ and $11B_6$ and the Fab′ monomer of either IgG or

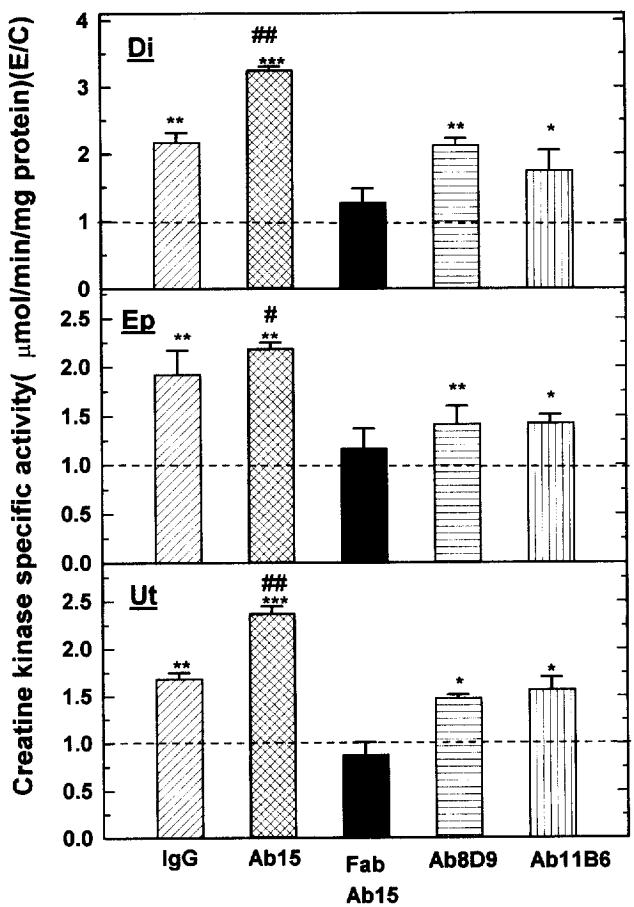

Fig. 1. Structural specificity of the anti-oestradiol antibody on enhancing the CK response to E_2 *in vivo*. Ovariectomized female rats were injected with mouse IgG_{2b} (mouse IgG, 25 μg/rat), E_2 (5 μg/rat), anti-E2 15 (25 μg/rat), anti-E2 $11B_6$ (25 μg/rat), anti-E2 $8D_9$ (25 μg/rat), the Fab′ of anti-E2 15 (25 μg/rat) or anti-oestradiol antibody plus E_2 (clone 15, $11B_6$ or $8D_9$). The animals were killed 24 h later, the various organs removed and the CK specific activity assayed. Di, diaphysis; Ep, epiphysis, Ut, uterus. Results are expressed as mean ± S.E.M. of the ratios of experimental/control (E/C) where the control is the value of the various treatments in the absence of E_2 for $n = 5$–10. *$P < 0.05$, **$P < 0.01$ for the statistical significance compared to untreated control group. ##For the statistical significance between Ab15 + E_2 vs E_2.

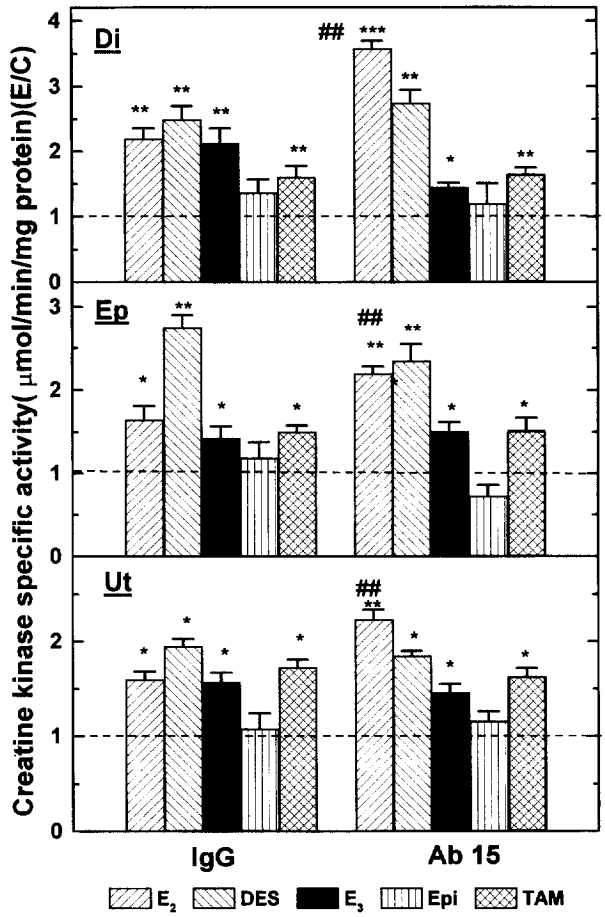

Fig. 2. Effect of Ab15 on enhancing the CK response by various oestrogenic mimetics. Ovariectomized female rats were injected with E2 (5 μg/rat), oestriol (E3, 5 μg/rat), diethystilbestrol (DES, 5 μg/rat), epiestriol (Epi, 5 μg/rat), tamoxifen (Tam, 500 μg/rat), mouse IgG2b (IgG, 25 μg/rat), anti-E2 15 (25 μg/rat) or anti-E2 15 plus DES, plus E_3, plus E_2, plus Epi or plus Tam. The CK activity of the various organs was assayed after 24 h. Di, diaphysis; Ep, epiphysis; Ut, uterus. Results are expressed as mean ± S.E.M. of the ratios of experimental/control (E/C) where the control is the value of IgG_{2b} or Ab15 injected alone for $n = 5$–10. *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$ for the statistical significance compared to the untreated group. ##For the statistical significance between anti-E2 15 + E_2 vs E_2.

anti-E_2 15 were not effective in enhancing the CK response to oestrogen. Moreover, the Fab′ of clone 15 inhibited the stimulation caused by oestradiol, indicating that the Fab′ monomer acts as a neutralizing antibody or antagonist in this system. At this concentration of the Fab′ of clone 15 was not capable of blocking the enhancing CK response to oestrogen plus anti-E_2 15 (date not shown).

The effect of anti-E_2 15 in potentiating the CK response by various oestrogenic mimetics

Ovariectomized female rats were injected with oestradiol, oestriol, epi-oestriol, tamoxifen, diethylstilbestrol in the presence of anti-E_2 15 or mouse IgG_{2b}.

Only the combination of anti-E_2 15 with oestradiol potentiated the CK response to oestrogen in the diaphysis, epiphysis and uterus. Oestriol, tamoxifen and diethylstilbestrol by themselves or with IgG_{2b} stimulated the CK specific activity in all these organs whereas epi-oestriol was devoid of activity (Fig. 2). It can be noted that although anti-E_2 15 cross-reacts with oestriol to a large extent (60%, see Table 1), the combination of anti-E_2 15 with oestriol did not lead to a potentiation of CK response to oestriol (Fig. 2). In addition, tamoxifen when administered with anti-E_2 15 plus E_2, acted like an antagonist and blocked the stimulatory CK response to anti E_2 plus E_2 (data not shown).

On the other hand, in the pituitary, anti-E_2 15 when given together with E_2, not only did not enhance, but abolished the stimulatory CK response

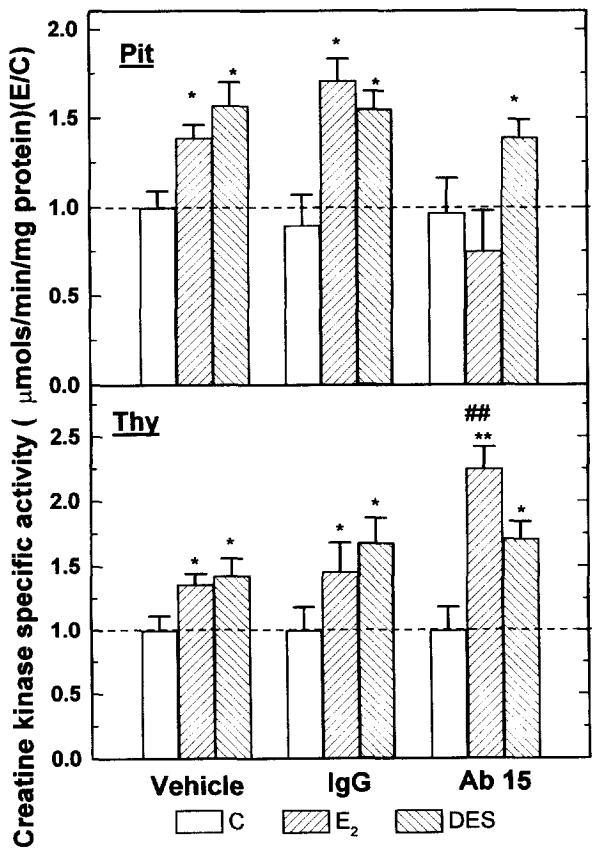

Fig. 3. Effect of anti-E_2 15 on potentiating the CK response to E_2 or DES in the pituitary and thymus of ovariectomized female rats. Ovariectomized female rats were injected with E_2 (5 μg/rat), DES (5 μg/rat), ethanol (Control, C), mouse IgG_{2b} (IgG) (25 μg/rat), anti-E_2 15 (25 μg/rat) or anti-E_2 plus E_2 or DES or mouse IgG_{2b} plus E_2 or DES. The CK activity in the pituitary (Pit) and thymus (Thy) was assayed 24 h after treatment. Results are expressed as mean ± S.E.M. of the ratios of experimental/control (E/C) where the control is the value of IgG_{2b} or Ab15 injected alone for $n = 5$ to 10. *$P < 0.05$, **$P < 0.01$; for the statistical significance compared to the untreated group. ##For the statistical significance between anti-E2 15 + E_2 vs. E_2.

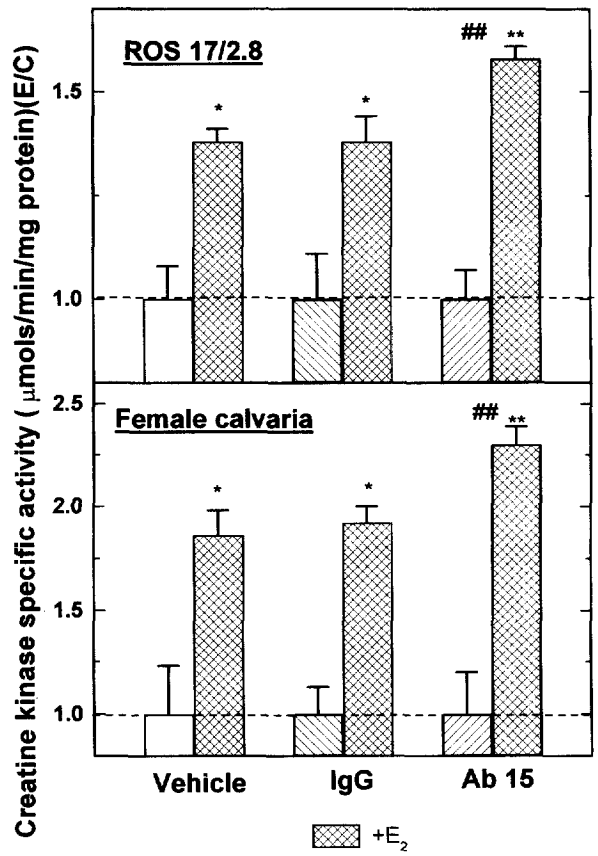

Fig. 4. Effect of treatment of anti-E_2 plus E_2 on CK activity *in vitro*. ROS 17/2.8 or rat embryo female calvaria cells were treated for 24 h with vehicle, irrelevant mouse IgG_{2b} (IgG, 2.5 μg/ml), E_2 (30 nM), Ab15 (2.5 μg/ml), a combination of IgG_{2b} plus E_2 or anti-E2 plus E_2. The CK specific activity was measured 24 h after the various treatments and expressed as experimental over control (E/C). *$P < 0.05$, **$P < 0.01$; for the statistical significance compared to the untreated group. ##For the statistical significance between anti-E2 15 + E_2 vs. E_2.

to E_2 in this organ, whereas the stimulatory CK response to DES was not affected (Fig. 3). These results are in accord with our previous work [3] where anti-E_2 15 when injected to cycling female rats on the morning of di-estrus, due to specific immunoneutralization of estradiol, completely blocked the LH surge on the afternoon of pro-estrus. Interestingly, in the thymus, anti-E_2 plus E_2 led to a potentiation of CK response to E_2 and not to DES (Fig. 3). Mouse IgG_{2b} was not effective in causing a potentiation of CK response to E_2 in the thymus (Fig. 3).

The effect of treatment with anti-E_2 on the CK response to oestradiol in cultured skeletal cells

When cultured rat embryo female derived calvaria cells or ROS 17/2.8 cells were incubated with E_2 (5 μg) plus anti-E_2 15 (2.5 μg/ml) there was a significant potentiation of CK response to E_2 (Fig. 4). Mouse IgG_{2b} used as control did not enhance the

CK response to E_2 in both cell types, as compared to E_2 alone.

CONCLUSIONS

The studies reported in this paper describe that only anti-E_2 15 and not clones $8D_9$ and $11B_6$ can potentiate the CK response to E_2 in oestrogen sensitive rat organs (diaphysis, epiphysis, uterus and thymus) *in vivo* (Fig. 1) and in skeletal derived cells *in vitro* (Fig. 4). The enhanced CK response was dose (Table 1) and time-dependent (Table 2) in the diaphysis, epiphysis and uterus. The pituitary did not respond to an enhanced CK activity to E_2 when the ovariectomized rats were treated with anti-E_2 15 plus E_2, indicating that the antibody could not penetrate the blood barrier in this organ (Fig. 3). On the contrary, the CK response to E_2 was blocked, indicating that anti-E_2 acted as an immunoblocker in this system. Moreover, treatment of ovariectomized rats with anti-E_2 15 plus DES did not effect the stimulatory CK response to DES in the pituitary (Fig. 3). These results confirm our previous report showing that anti-E_2 15 blocks E_2 induced LH surge [3].

The enhancing effect of anti-E_2 15 on the CK response by E_2 in rat organs was observed only when anti-E_2 15 was injected simultaneously with E_2 and not with oestriol (E_3), tamoxifen, epi-oestriol or diethylstilbestrol (see Fig. 2), suggesting that the observed enhancing effect was specific only to the simultaneous administration of anti-E_2 15 plus E_2. Interestingly, E_3 by itself is stimulatory and anti-E_2 15 cross-reacts with E_3 to a large extent (see Table 1). Moreover, the stimulatory CK response to anti-E_2 15 + E_2 in these organs is completely blocked by the anti-oestrogen, tamoxifen (Fig. 2) and not by the Fab′ monomer of anti-E_2 15, which blocks only E_2 effect.

All three clones show high affinity to oestradiol (see Table 1). However, when the various anti-E_2 antibodies, clones 15, $8D_9$ and $11B_6$, respectively, were compared in terms of potentiating CK response to E_2, only anti-E_2 15 was capable in enhancing the CK response to E_2 in the uterus, diaphysis and epiphysis (see Fig. 1), suggesting the structural diversity of the three anti-E_2 antibodies in eliciting an enhanced CK response to E_2 in rat organs.

There are several studies in the literature on enhancing antibodies such as anti-cytokine [15] and anti-human growth hormone antibodies [16]. To our knowledge, this is the first report of an anti-steroidal antibody that can potentiate the biological effect of the hormone oestradiol. It can be speculated that the binding site of anti-E_2 15 accommodates E_2 easily, and the anti-E_2 15 with E_2 present in its binding site may serve as a specific carrier that can deliver E_2 to the oestrogen sensitive target organs, resulting in an enhancement of CK activity. Along these lines we noticed in our previous work that we could block ovulation with anti-E_2 15 without affecting the uterine ballooning of cycling female rats, indicating that oestrogen was present in this tissue (Kohen and Fajer, unpublished). We suggest that anti-E_2 15 may be a useful tool in enhancing the biological effects of E_2 both *in vivo* and *in vitro*, and its Fab′ fragment may be used as an antagonist.

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