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Legends for Figures

Figure 1. Neurosteroid-induced -HEX release from RBL-2H3 cells and its blockade by PROG and EDCs. RBL-2H3 cells were incubated with 30 M quercetin for 48 h in normal growth medium. Data represent the mean ± S.E. from more than 3 separate experiments. *p<0.05, compared with the vehicle-treated group. (*A*) Time course of DHEAS-induced -HEX release. Confluent cultures in 24-well plates were stimulated with DHEAS (1 M) and -HEX release was evaluated using supernatants from the cells at the indicated periods. The results represent the ratio (%) of -HEX release to the total content. (B) Degranulation by several neurosteroids. The results represent the -HEX release (%) by various neurosteroids (1 M). (C) Antagonistic effects of PROG, E_2 and their BSA-conjugates (10 M) on DHEAS- and PREGS (1 M)-induced -HEX release. (D) Competitive blockade of DHEAS-induced degranulation by PROG-BSA (10 M). (E) Lack of -HEX release by EDCs (1 M). (F) Antagonism of DHEAS-induced -HEX release by EDCs (10 M).

Figure 2. Gq/11 and PLC activation-mediated -HEX release by DHEAS. The results represent the mean ± S.E. from more than 3 separate experiments. *p<0.05, compared with the DHEAS- or PREGS-treated group. (A) Effects of several inhibitors on DHEAS (1 M)-induced -HEX release. PTX, U-73343 and U-73122 were used at 10 M. Xestospongin C, wortmannin, RU-486 (RU) and ICI-182,734 (ICI) were used at 1 M. EGTA was used at 1 mM. (B) Effects of the $G_{q/11}$ AS-ODN on DHEAS (1 M)-induced -HEX release. Inset: Reduction in the $G_{q/11}$ level assessed by Western blotting analysis (2 g protein per lane, 12% acrylamide gel). Results represent

DHEAS-induced -HEX release (%) using RBL-2H3 cells pretreated with AS-ODN and MS-ODN for $G_{q/11}$. (C) No effect of oxytocine on DHEAS-induced -HEX release. Oxytocin (10 M) was added with or without DHEAS (1 M). (D) No effect of DHEAS (1 M)-induced -HEX release by 25-DX AS-ODN. Inset: Reduction in the 25-DX level by AS-ODN or MS-ODN in Western blotting analysis (1.5 g protein per lane, 12% acrylamide gel). Veh: vehicle-, AS; AS-ODN-, MS; MS-ODN-treatment.

Figure 3. PROG-BSA-FITC binding to RBL-2H3 cells. PROG-BSA-FITC (1 M) binding to RBL-2H3 cells was performed in the absence or presence of PROG (10 M), DHEAS (10 M) or NP (10 M). The details are described in the Materials and Methods.

Figure 4. DHEAS-induced nocifensive responses. The results represent the mean ± S.E. of the total period (s) showing nocifensive biting and licking behaviors from more than 3 separate experiments. (A) Dose-dependent DHEAS (1-10 fmol)-induced nocifensive behaviors. *p<0.05, compared with the vehicle-treated group. (B) Blockade of DHEAS (3 fmol, i.pl.)-induced nocifensive responses by co-administration of PROG-BSA (1-30 fmol, i.pl.). *p<0.05, compared with the DHEAS (10 fmol, i.pl.)-treated group. (C) Blockade of DHEAS-induced nocifensive responses by co-administration of Progesterone (PROG) or Nonyphenol (NP) at 10 fmol (i.pl.). † p<0.05, compared with the group without PROG-BSA. (D) No effect of oxytocien (10 fmol) on DHEAS (3 fmol)-induced nocifensive responses. *p<0.05, compared with the vehicle-treated group.

Fig.1

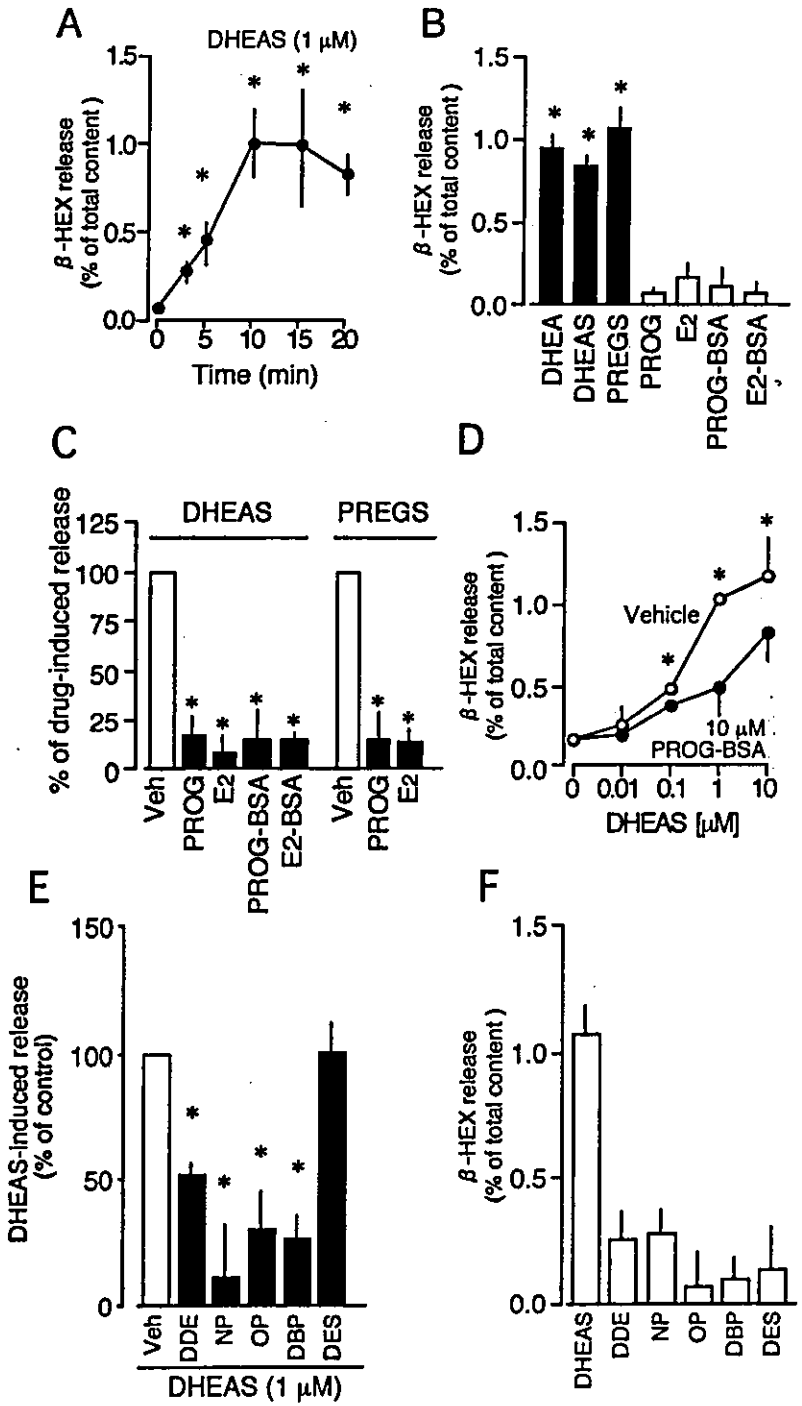

A
DHEAS (1 μM)
β-HEX release (% of total content)
Time (min)
B
DHEA
DHEAS
PREGS
PROG
E2
PROG-BSA
E2-BSA
C
DHEAS
PREGS
% of drug-induced release
Veh
PROG
E2
PROG-BSA
E2-BSA
D
Vehicle
10 μM PROG-BSA
DHEAS [μM]
E
DHEAS-induced release (% of control)
Veh
DDE
NP
OP
DBP
DES
DHEAS (1 μM)
F
DHEAS
DDE
NP
OP
DBP
DES

Fig.2

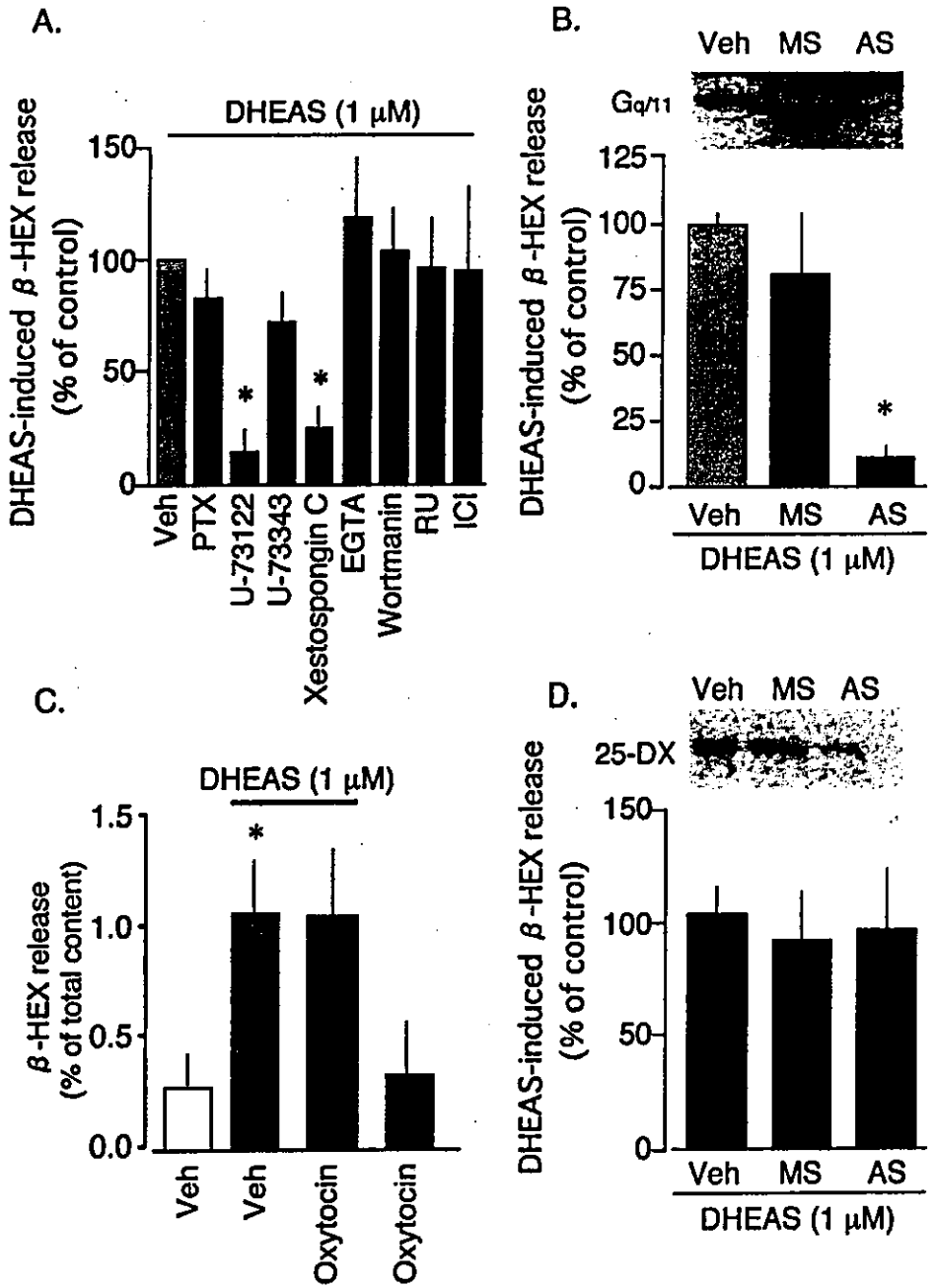

A.
DHEAS (1 μM)
DHEAS-induced β-HEX release (% of control)
150
100
50
0
*
*
Veh
PTX
U-73122
U-73343
Xestospongin C
EGTA
Wortmanin
RU
ICI
B.
Veh MS AS
Gq/11
DHEAS-induced β-HEX release (% of control)
125
100
75
50
25
0
*
Veh MS AS
DHEAS (1 μM)
C.
DHEAS (1 μM)
β-HEX release (% of total content)
1.5
1.0
0.5
0.0
*
Veh
Veh
Oxytocin
Oxytocin
D.
Veh MS AS
25-DX
DHEAS-induced β-HEX release (% of control)
150
100
50
0
Veh MS AS
DHEAS (1 μM)

Fig.3

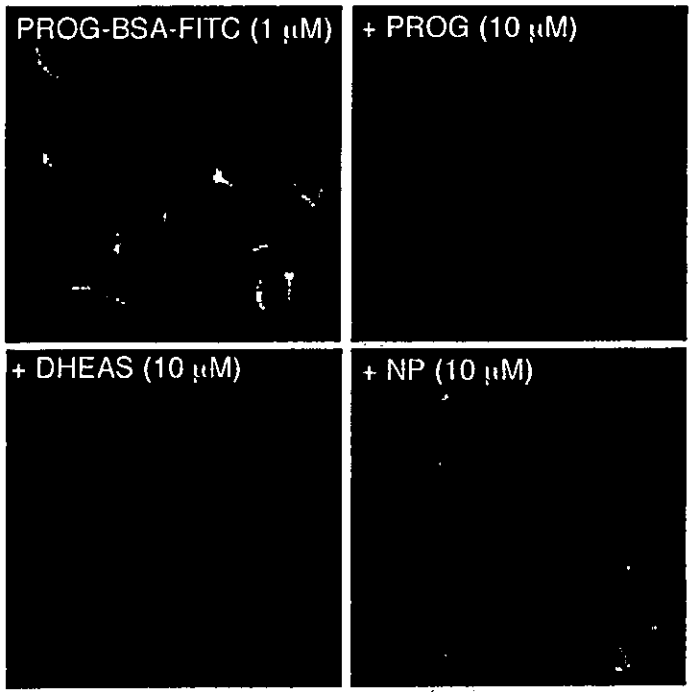
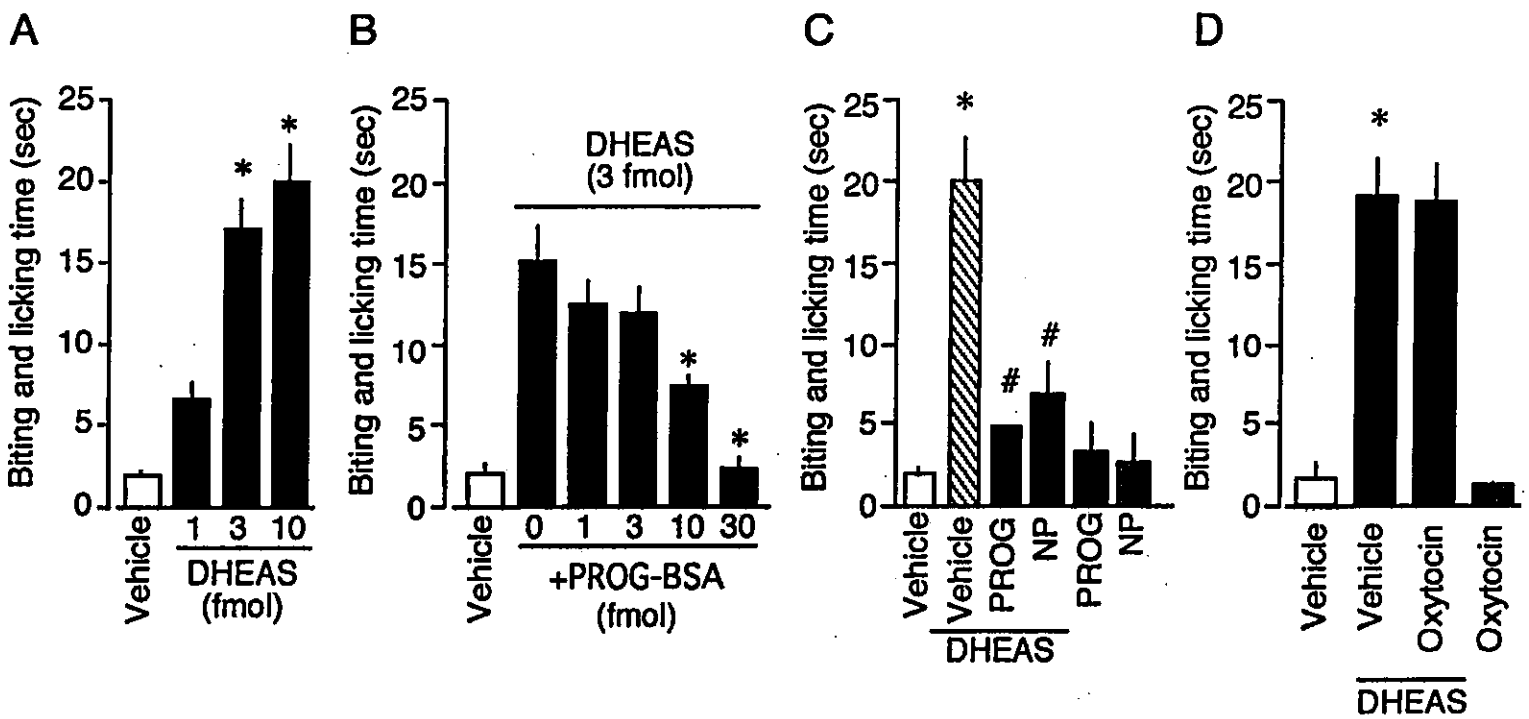


Fig.4


A
B
C
D
Biting and licking time (sec)
25
20
15
10
5
0
Vehicle
1 3 10
DHEAS
(fmol)
DHEAS
(3 fmol)
0 1 3 10 30
+PROG-BSA
(fmol)
Vehicle
PROG
NP
PROG
NP
DHEAS
Oxytocin
Oxytocin
*
#

Supplementary data