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Identification of β-adrenoceptors in mouse myometrium by radioligand binding study

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ABSTRACT The binding of radioligand to cell membrane prepared from mouse myometrium was used to identify the characteristics of β-adrenoceptors. Uteri were taken from mature mice (Kunming Strain) weighing 10±5 g pretreated with estradiol benzoate 1 mg·kg^{-1}·d^{-1} ip for 2 d. The binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA) to uterine membrane was saturable, having a B_{max} of 378 fmol/mg protein and a K_D of 3.1 nmol·L^{-1}. The slope of Hill plot (n_H = 1.03) indicated the absence of cooperative interactions. IC_{50} and K_i values of *l*-norepinephrine (NE), *dl*-propranol (Pro), *d*-timolol (*d*-Tim), and *l*-Tim competed for the [^{3}H]DHA binding sites were 171±10, 10.3±4.3, 6.5±2.1, 0.40±0.23 nmol·L^{-1} and 113±6, 6.3±2.8, 4.0±1.3, 0.25±0.14 nmol·L^{-1}, respectively. These 4 agents competed for the [^{3}H]DHA binding sites in an order of potency: *l*-Tim > *d*-Tim > Pro > NE. Atenolol (Ate) did not compete for [^{3}H]DHA binding sites. The results suggested that these binding sites for [^{3}H]DHA in mouse myometrium appeared to be β_2-adrenoceptor.

KEY WORDS myometrium; norepinephrine; propranolol; timolol; atenolol; beta adrenergic receptors; dihydroalprenolol; radioligand assay

Although adrenoceptors have been identified in uterine smooth muscle of some laboratory animals[1-2] and human[3] by radioligand binding studies, there has been little information about the nature of adrenoceptors in mouse uterus. Previous studies in our laboratory showed the existence of adrenoceptors in mouse myometrium by analysis of the actions of adrenoceptor agonists and blockers on the contraction of isolated uterine strips and the myometrial cAMP levels[4]. In this report, we identified the adrenoceptors in mouse myometrium using the labeled adrenoceptor blocker, [^{3}H]dihydroalprenolol ([^{3}H]DHA).

MATERIALS AND METHODS

Drugs Atenolol (Ate) was synthesized by Shanghai Second Pharmaceutical Factory, propranolol (Pro) was provided by Shanghai Huanghe Pharmaceutical Factory. d-Timolol (*d*-Tim) and *l*-timolol (*l*-Tim) were provided by Professor JIANG Ming-Hua. NE was purchased from Sigma Chemical Company, [^{3}H]DHA (1.7 PBq·mol^{-1}) was purchased from China Institute of Atomic Energy, Beijing.

Membrane preparation Uteri from mature mice (Kunming strain, 40±5 g) were stripped off fat, minced, and homogenized in ice cold buffer (sucrose 0.25 mol·L^{-1}, $MgCl_2$ 1 mmol·L^{-1}, and Tris 5 mmol·L^{-1}, pH 7.4). The homogenate was centrifuged at 500×*g* for 10 min at 4 ℃ and the pellet was discarded. The supernatant was centrifuged at 47 000×*g* for 15 min at 4 ℃. The resulting pellet was washed twice in ice cold incubation buffer (Tris 50 mmol·L^{-1} and $MgCl_2$ 10 mmol·L^{-1}, pH 7.5) by resuspension and centrifugation at 47 000×*g* for 10 min. The final pellet was resuspended in incubation buffer for use in the binding assay.

Binding assay [^{3}H]DHA (0.2—16 nmol·L^{-1}) and uterine cell membrane preparations were incubated at 25 ℃ for 15 min in duplicate tubes with and without a high concentration (10 μmol·L^{-1}) of Pro in a final

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volume of 1 ml. Incubation was terminated by a rapid dilution with 3 ml cold buffer followed by a rapid filtration through Whatman GFC glass fiber filter. Filters were rapidly washed with 9 ml of cold incubation buffer, dried at 105±5 ℃ for 30 min and counted in 5 ml of dimethyl benzene solution including PPO (20 mg) and POPOP (0.5 mg) in a liquid scintillation counter (Beckman LS 8000) at an efficiency of 53 %. Specific binding was taken as the difference between radioactivities measured on the filters in the absence and presence of Pro. Equilibrium dissociation constant (K_D) and maximal number of binding sites (B_{max}) were determined by Scatchard analysis using linear regression.

Statistics IC_{50} was determined by simplified probit method with 95 % confidence limit. K_i values were calculated according to Cheng-Prusoff equation.

RESULTS

Number and affinity of binding sites

The binding of [^{3}H]DHA to mouse uterine cell membrane was found to be a concentration-dependent and saturable process (Fig 1). The Scatchard plot showed a K_D value of 3.1 ±1.2 nmol·L^{-1} and B_{max} of 378±74 fmol·L^{-1}/mg protein (n=4). The slope of Hill plot indicated the absence of cooperation interactions (Fig 2).

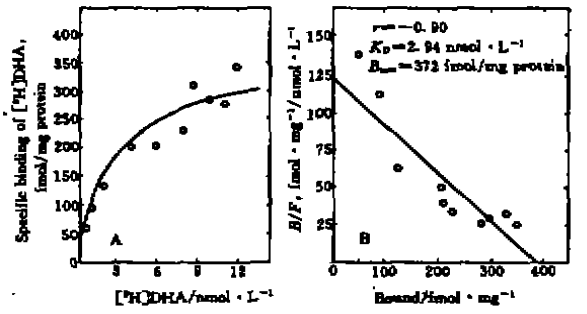

Fig 1. A representative of specific binding of [^{3}H]dihydroalprenolol to binding site in mouse myometrial cell membrane (A) and Scatchard plot (B). $\bar{x}$=mean of duplicate determinations.

Specificity of binding Adrenergic agents competed for the [^{3}H]DHA binding sites in an order of potency: *l*-Tim>*d*-Tim> Pro> NE (Tab 1). The difference in the abilities of *l*-Tim and *d*-Tim to displace [^{3}H]DHA binding clearly showed the stereoselective nature of this binding. The results demonstrated that these binding sites for [^{3}H]DHA were β-adrenoceptors. The selective β_1-adrenoceptor blocker, Ate did not compete for [^{3}H]DHA binding sites as the non-selective adrenoceptor blockers.

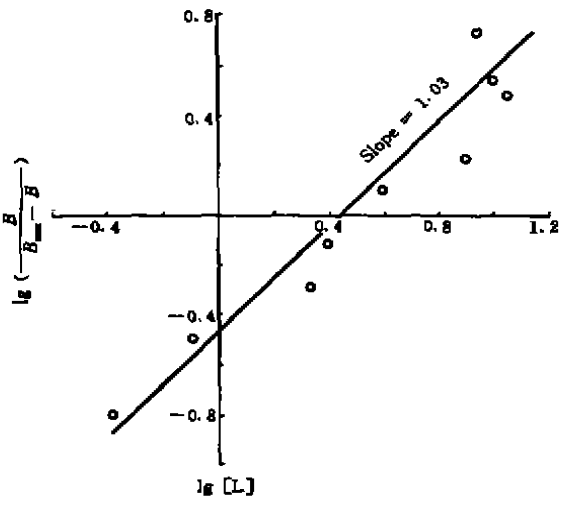

Fig 2. Hill plot of [^{3}H]DHA binding to mouse myometrial cell membrane.

Tab 1. IC_{50} and K_i values for competitive inhibitory effects of 4 adrenoceptor agonists and antagonists on [^{3}H]dihydroalprenolol binding in mouse myometrial cell membrane. n=4. $\bar{x}\pm s$.

Drug	IC_{50}/nmol·L^{-1}	K_i/nmol·L^{-1}
l-norepinephrine	171±10	113±8
dl-propranolol	10.3±1.3	6.3±2.8
d-timolol	6.5±2.1	4.0±1.3
l-timolol	0.10±0.23	0.25±0.11

DISCUSSION

In this paper, we adopted the labeled β-adrenoceptor blocker [^{3}H]DHA as radioligand

to bind with mouse uterine smooth muscle membrane. The binding was saturable, structurally specific and stereospecific. The results suggested these mouse uterine binding sites identified with [^{3}H]DHA appeared to have the charateristics of physiological β-adrenoceptors. The results that the selective β_1 blockers, Ate did not competed for [^{3}H]DHA binding site as the 3 non-selective blockers did and the slope of the Hill plot approached 1, which implied the ligand binding site being one[5], indicated that β-adrenoceptors in the mouse myometrium were mainly of β_2 subtype. Previous studies by analyzing the actions of adrenoceptor agonists and blockers on the contraction of isolated mice uterine strips in our laboratory also showed the predominance of the β_2 subtype[6,7].

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放射配体结合法鉴定小鼠子宫平滑肌β肾上腺素受体

R965.2

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摘要 用放射配体结合法鉴定小鼠子宫平滑肌β肾上腺素受体，结果显示 *l*-去甲肾上腺（NE），*dl*-普萘洛尔（Pro），*d*-噻吗洛尔（*d*-Tim），*l*-Tim 的 IC_{50} 依次为171±10，10.3±4.3，6.5±2.1，0.40±0.23 nmol·L^{-1}；K_I 依次为113±6，6.3±2.8，4.0±1.3，0.25±0.14 nmol·L^{-1}，亲合力顺序为 *l*-Tim>*d*-Tim>*dl*-Pro>*l*-NE，阿替洛尔不能抑制[^{3}H]二氢阿普丙洛尔的结合，提示小鼠子宫平滑肌具有β_2肾上腺素受体.

关键词 子宫肌层；去甲肾上腺素；普萘洛尔；噻吗洛尔；阿替洛尔；β肾上腺素受体；二氢阿普洛尔；放射配体测定