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Leukotriene C_4 receptors in cultured smooth muscle cells from bovine anterior cerebral arteries and microcerebrovasculatures[1]

ZENG Guo–Qian, RUI Yao–Cheng, SUN Du–Xin, SHEN You–An
(*Department of Pharmacology, School of Pharmacy, Second Military Medical University, Shanghai 200433, China*)

ABSTRACT Specific receptors for leukotriene C_4 (LTC_4) were identified on smooth muscle cells isolated from bovine anterior cerebral arteries (BACASMC) and bovine microcerebrovasculatures (BMSMC). [^{3}H]LTC_4 specific bindings to both cells at a fixed input reached the maxima at 60 min and 20 min, respectively. With incremental inputs of radioligand and a constant cell number, [^{3}H]LTC_4 specific bindings reached a plateau indicative of a saturable binding site. Analysis of Scatchard plots demonstrated a

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single population of high-affinity binding sites in both cells. The dissociation constant (K_d) for BACASMC was 39.2 ± 1.3 nmol · L^{-1} and its B_{max} was 19.3 ± 2.1 pmol / 10^6 cells. For BMSMC, K_d = 2.0 ± 0.4 nmol · L^{-1}, B_{max} = 157 ± 13 fmol / 10^6 cells. The specific [^{3}H]LTC_4 bindings was inhibited by unlabeled LTC_4, LTD_4 and FPL-55712 (an SRS-A antagonist). The inhibitory rates for BACASMC were 70.4% and 35.3% by LTC_4 and FPL-55712 at 1 μmol · L^{-1}, respectively. For BMSMC the inhibitory rates were 96.9%, 73.9%, and 44.9% by LTC_4, LTD_4, and FPL-55712 at 10 μmol · L^{-1}, respectively.

KEY WORDS SRS-A; drug receptors; vascular smooth muscle; radioligand assay; cattle; brain

SRS-A, a metabolite of arachidonic acid, is an important chemical mediator which participates in the pathogenesis of many diseases[1]. Its biological effect is thought to be brought about by specific receptors, and the stereospecific binding sites of SRS-A have been found in several peripheral tissues[2,3] and central nervous system[4,5]. SRS-A constricts cerebral artery[6] and plays an important role in the development of vasogenic brain edema[7]. These effects are also thought to be receptor-mediated. In this study we investigated whether specific receptors for leukotriene C_4 (LTC_4) were present in cultured bovine anterior cerebral arterial and microcerebrovascular smooth muscle cells.

MATERIALS AND METHODS

Reagents [^{3}H]LTC_4 (1 420.8 TBq / mol) was purchased from New England Nuclear Co, Boston MA, USA. Synthetic LTC_4 was kindly gifted by the Merck Frosst Co, Canada. FPL-55712 was supplied by Fisons Ltd, Loughborough, UK.

Reaction solution The binding assays were done in the reaction solution of Tris-HCl, pH 7.4, containing serine 5, borate 10, $MgCl_2$ 10, and Tris 50 mmol · L^{-1}.

Cell culture The bovine anterior cerebral arterial and microcerebrovascular smooth muscle cells *in vitro* were established according to Ref 8 and Ref 9. The following experiments were performed using the 4th-5th generations of the cells.

[^{3}H]LTC_4 binding assay Freshly digested BACASMC were implanted in a 24-well culture plate (5×10^4 cells / well). The medium was discarded 3 d later and the cells were washed twice with reaction solution. Then another 0.5 ml reaction solution with varying concentrations of [^{3}H]LTC_4 (0.25-13.5 nmol · L^{-1}) for saturation experiments or with fixed amounts of [^{3}H]LTC_4 (2 nmol · L^{-1}) for competition study was added. For nonspecific binding unlabeled LTC_4 (2000 times more than [^{3}H]LTC_4) was added to each well. Incubation continued for 60 min at 4℃. For BMSMC, the binding assay was performed at 25℃ for 20 min. The cells were filtered under vacuum through separate glass-fiber filters on a millipore (0.22 μm) filtration manifold at the end of reaction. Each filter was washed 5 times with 2-ml portions of cold reaction solution, and then placed into a scintillation vial with 0.2 ml cocktail for scintillation counting. Total and nonspecific binding of [^{3}H]LTC_4 were determined as the mean of triplicate assays carried out in the absence or presence of unlabeled LTC_4. Specific binding was calculated as the difference between total and nonspecific bindings at each concentration pf [^{3}H]LTC_4. Binding data were analyzed by the Scatchard plots.

RESULTS

Kinetic analysis The association studies were made by using [^{3}H]LTC_4 1.5 nmol · L^{-1} for total binding and unlabeled LTC_4 3 μmol · L^{-1} for nonspecific binding. Under these conditions, the specific [^{3}H]LTC_4 binding to BACASMC increased rapidly over the first 10 min of incubation, and gradually progressed to equilibrium at 60 min and remained stable at 120 min. The binding to BMSMC reached the maximum at 20 min. Nonspecific binding thereafter was independent of time.

Saturation experiments With increasing concentrations of radioligand and 2×10^5 smooth muscle cells, specific binding of

[^{3}H]LTC_4 to both cells was saturable in a concentration-dependent manner (Fig 1). Nonspecific binding increased progressively up to maximal input assessed. Scatchard plots obtained from 3 experiments indicated a single population of the specific [^{3}H]LTC_4 binding sites with a K_d of 39.2 ± 1.3 nmol · L^{-1} and B_{max} of 19.3 ± 2.1 pmol / 10^6 cells for BACASMC (Fig 1A inset). For BMSMC, K_d = 2.0 ± 0.4 nmol · L^{-1}, B_{max} = 157 ± 13 fmol / 10^6 cells (Fig 1B inset).

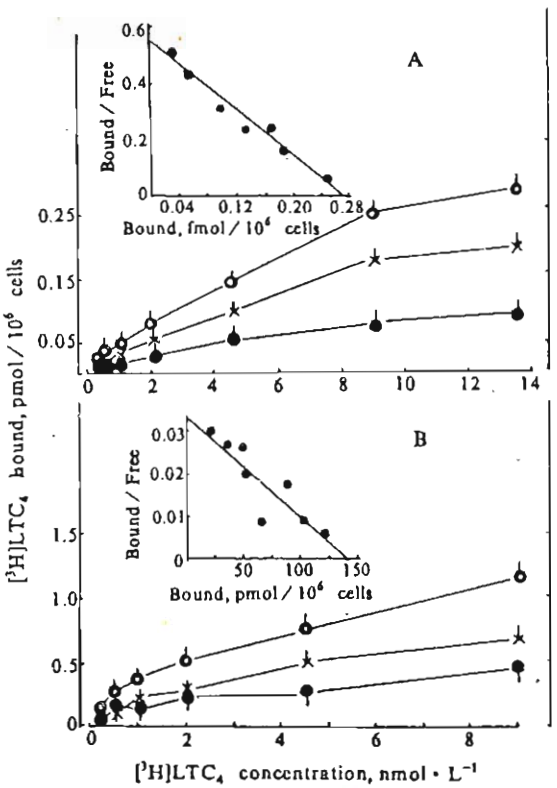

Fig 1. Scatchard plot of [^{3}H]LTC_4 binding to bovine anterior cerebral arteries (A) and microcerebrovasculatures (B). Total binding (○), nonspecific binding (●), and specific binding (×) of [^{3}H]LTC_4 as a function of incremental inputs of [^{3}H]LTC_4. n=3, $\bar{x} \pm s$.

Competition studies To evaluate the specificity of [^{3}H]LTC_4 binding sites, we used unlabeled LTC_4, LTD_4, and FPL-55712 to assess their capacities to inhibit the specific binding of [^{3}H]LTC_4. LTC_4 and FPL-55712 at 1 μmol · L^{-1} reduced respectively 70.4% and 35.3% specific [^{3}H]LTC_4 binding to BACASMC (Tab 1). At 10 μmol · L^{-1}, the specific binding to BMSMC were reduced 96.9%, 73.9%, and 44.9% by LTC_4, LTD_4, and FPL-55712, respectively (Tab 1).

Tab 1. Displacement of [^{3}H]LTC_4 binding to smooth muscle cells of bovine anterior cerebral arteries (BACASMC) and bovine cerebral microvasculatures (BMSMC) by unlabeled LTC_4, LTD_4, and FPL-55712. The cells were incubated with [^{3}H]LTC_4 2 nmol · L^{-1} in the absence or presence of LTC_4, LTD_4, and FPL-55712. n=3, $\bar{x} \pm s$. $^{***}P<0.01$ vs control.

Addition / μmol · L^{-1}		Bound / dpm BACASMC	Bound / dpm BMSMC
none	0	2 373 ± 154	965 ± 83
LTC_4	1	702 ± 85***	393 ± 32***
LTC_4	10		30 ± 5***
LTD_4	10		250 ± 37***
FPL-55712	1	1 535 ± 94***	
FPL-55712	10		532 ± 64***
FPL-55712	30	410 ± 37***	

DISCUSSION

Specific binding stites of LTC_4 have been demonstrated recently on a variety of smooth muscle cells *in vitro*[11-13]. In this report, the specific binding sites of [^{3}H]LTC_4 were identified in bovine anterior cerebral arterial and microcerebrovascular smooth muscle cells *in vitro*. Specific binding to both smooth muscle cells was rapid, saturable, and reversible. The K_d and B_{max} values were consistant with another report[2]. Our previous work showed that LTC_4 stimulated TXA_2 synthesis in bovine anterior cerebral arterial smooth muscle cells in culture[10] and that LTC_4 promoted the proliferation and DNA synthesis of the above cells and bovine cerebral microvascular smooth muscle cells (data not

show). These effects might be mediated by specific LTC_4 receptors reported here in the smooth muscle cells.

There were some evidence which implicated SRS-A in the development of vasogenic brain edema[7]. From the present studies we also speculated that the effects of SRS-A on brain edema might be receptor-mediated and that these LTC_4 receptors are important in regulating brain-blood barrier permeability and in the development of cerebral arthero-sclerosis.

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培养的牛脑前动脉及微血管平滑肌细胞上白三烯 C_4 受体

曾国钱、芮耀诚、孙笃新、沈有安 (第二军医大学药学院药理教研室，上海 200433，中国)

提要 [^{3}H]LTC_4 与牛脑前动脉及微血管平滑肌细胞的结合具有特异、饱和及可逆的特点. 在前一种细胞上，$K_d = 39.2 \pm 1.3$ nmol · L^{-1}，$B_{max} = 19.3 \pm 2.1$ pmol / 10^6 细胞，LTC_4 和 FPL-55712 在 1 μmol · L^{-1} 时对[^{3}H]LTC_4 结合的取代率分别为 70.4%和 35.3%. 在后一种细胞上，$K_d = 2.0 \pm 0.4$ nmol · L^{-1}，$B_{max} = 157 \pm 13$ fmol / 10^6 细胞，LTC_4，LTD_4 和 FPL-55712 在 10 μmol · L^{-1} 的取代率分别为 96.9%，73.9%和 44.9%.

关键词 慢反应物质 A；药物受体；血管平滑肌；放射配位体测定；牛；脑