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东莨菪碱对小鼠被动学习、探究行为及脑区毒蕈碱受体的昼夜变化

潘思源 R926

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提要 东莨菪碱(Scop) ip 0.1，0.4 mg · kg^{-1}. 实验于 07:00–09:00，15:00–17:00 和 21:00–23:00 进行. 结果显示，Scop 对小鼠被动学习的抑制作用，及其增加探究活动和减少排便的作用，以白天最明显. 小鼠额叶皮层和海马部位的 M 受体数，白天多于夜晚. 而纹状体 M 受体以上午最少. 结果提示，Scop 对小鼠学习记忆和行为的影响，及其不同脑区的 M 受体，均呈现一定的昼夜变化.

关键词 东莨菪碱；二苯羟乙酸奎宁酯；记忆；探究行为；毒蕈碱受体；额叶；纹状体；海马；昼夜节律

BIBLID: ISSN 0253–9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; **13** (4) : 326–329

Specific binding sites of platelet activating factor on the intact bovine cerebral microvascular endothelial cells and antagonism of drugs

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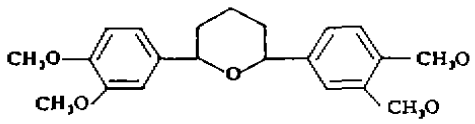
ABSTRACT [^{3}H]Triazolodiazepine ([^{3}H]WEB 2086), an antagonist of platelet activating factor (PAF) receptor, was studied as radioligand on intact cerebral microvascular endothelial cells (CMEC). The results showed that the binding of [^{3}H]triazolodiazepine reached and maintained at an equilibrium after 15–120 min of incubation and that it was saturable with increasing concentration of radioligand. Scatchard analysis indicated that there were 2 specific binding sites on CMEC, its K_{d1}, B_{max1}, K_{d2}, and B_{max2} were 3.13 nmol · L^{-1}, 1.50 pmol / 3 × 10^5 cells, 83.96 nmol · L^{-1}, and 12.96 pmol / 3 × 10^5 cells, respectively. The binding of [^{3}H]triazolodiazepine to CMEC was displaced by C_{16}–PAF and 1,5–bis–(3,4–dimethoxyphenyl)–tetrahydro–(4*H*)–pyran (SZ–1), which IC_{50} were 0.43 nmol · L^{-1} and 0.125 μmol · L^{-1}, respectively. These data suggested the existence of PAF specific binding sites on CMEC.

Received 1991 Apr 15 Accepted 1992 May 4

KEY WORDS platelet activating factor; vascular endothelium; drug receptors

Platelet activating factor (PAF) is a potent phospholipid mediator involved in various inflammatory[1], respiratory[2], cardiovascular[3], and renal disorders[4]. PAF was synthesized in bovine brain, and it dose-depently constricted the pial artery of piglets[5,6]. PAF receptor antagonists, kadsurenone and ginkgolides, dose-dependently enhanced the recovery after cerebral ischemia[7,8].

However, the specific binding sites of PAF on the cerebrovascular system especially on the cerebral microvascular endothelial cells (CMEC) have not been described. In the present study, we studied the specific binding sites of PAF on CMEC with PAF receptor antagonist [^{3}H]triazolodiazepine ([^{3}H]WEB 2086) and the antagonism of a new compound 1,5-bis-(3,4-dimethoxyphenyl)-tetrahydro-(4*H*)-pyran (SZ-1) synthesized in our Department.



1,5-bis-(3,4-dimethoxyphenyl)-tetrahydro-(4*H*)-pyran (SZ-1)

MATERIALS AND METHODS

Minimum essential medium (MEM) (Gibco), trypsin (Sigma), [^{3}H]triazolodiazepine, triazolodiazepine, and C_{16}-PAF were gifted from Boehringer Ingelheim, Germany.

Cultivation of bovine CMEC Cell cultivation was prepared by a modification of the method[9]. In brief, the gray matter of bovine brain was collected, rinsed 4 times with D-Hank's solution, and cut into small pieces. Then the gray matter was homogenized 20 up and down strokes in a glass homogenizer with MEM. The homogenate was filtered through a nylon mesh with pore size of 74 μm. Microvessels were collected by the nylon mesh, and washed by centrifugation (800 × *g* for 8 min) with 10 ml MEM. The pellet was resuspended in 0.1% collagenase and incubated for 30 min at 37 ℃. At the end of incubation, the suspension was centrifugated (800 × *g* for 8 min) and the pellet resuspended in MEM with 20% fetal bovine serum and plated onto gelatin-coated tissue culture flask. The cultures were kept 7 d at 37 ℃ with 5% CO_2, fed with fresh medium every 2-3 d. The cells were subcultured with 0.1% trypsin.

Binding assay The cells were collected with 0.1% trypsin, washed with tris-HCl buffer (KCl 140 mmol · L^{-1}, tris 10 mmol · L^{-1}, BSA 0.1%, pH 7.4) by centrifugation (800 × *g* for 8 min), the pellet were resuspended in tris-HCl buffer (6×10^5 cells / ml). The cells suspension (3×10^5 cells) were added to a tube containing [^{3}H]triazolodiazepine 8 nmol · L^{-1}, with or without 1000-fold unlabeled triazolodiazepine (for nonspecific binding), incubated at 25 ℃ for 15 min. Bound and free radioligand were separated by rapid filtration with microporous filtering film which was presoaked in tris-HCl buffer for 24 h. The filters were washed 4 times with 2 ml ice-cold tris-HCl buffer (no 0.1% BSA). The radioactivity of the filter was measured by scintillation with 40% efficiency.

RESULTS

The cells were characterized by phase-contrast microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and factor VIII-related antigen staining. The cells showed the characteristics of endothelial cells, such as cobblestone colonies under the phase-contrast microscope. Tight junctions and pinocytic vesicles were present under TEM. The staining of factor VIII-related an antigen were positive.

Kinetic experiments The specific binding of [^{3}H]triazolodiazepine (8 nmol · L^{-1}) to CMEC reached rapidly and maintained steadily at an equilibrium in 15-120 min at 25 ℃. The nonspecific binding was unchangeable during 15-120 min and amounted to only

50% of the total bound (Fig 1).

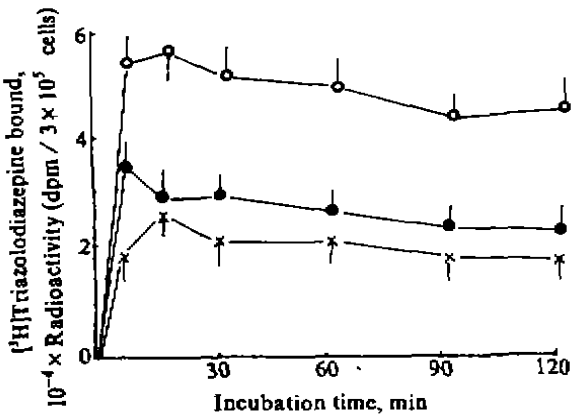


Fig 1. [³H]Triazolodiazepine binding to intact CMEC. Total bound (○), nonspecific bound (●), and specific bound (×). $n=3$, $\bar{x} \pm s$.

Saturation study The binding of [³H]triazolodiazepine to CMEC was specific and saturable with the increase of [³H]triazolodiazepine concentration (2–150 nmol · L^{-1}). The nonspecific binding increased linearly with radioligand concentration. Scatchard analysis indicated that there were 2 specific binding sites of [³H]triazolodiazepine on CMEC. Their K_{d1}, K_{d2}, B_{max1}, and B_{max2} were 3.13 nmol · L^{-1}, 83.96 nmol · L^{-1}, 1.50 pmol / 3×10^5 cells, and 12.96 pmol / 3×10^5 cells, respectively (Fig 2).

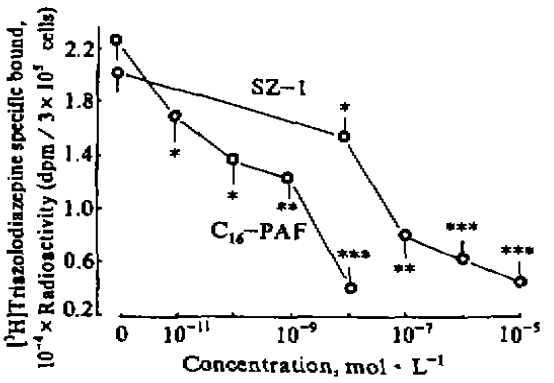


Fig 2. Saturation and Scatchard analysis of [³H]triazolodiazepine specific binding to intact CMEC. $n=3$, $\bar{x} \pm s$.

Competition study The specificity of [³H]triazolodiazepine binding to CMEC was established by displacing [³H]triazolodiazepine 8 nmol · L^{-1} by C_{16}–PAF and SZ–1 in competition experiments. C_{16}–PAF (0.01–10 nmol · L^{-1}) and SZ–1 (0.01–10 μmol · L^{-1}) dose–dependently inhibited the specific binding of [³H]triazolodiazepine to CMEC with IC_{50} values of 0.44 nmol · L^{-1} and 0.13 μmol · L^{-1}, respectively (Fig 3).

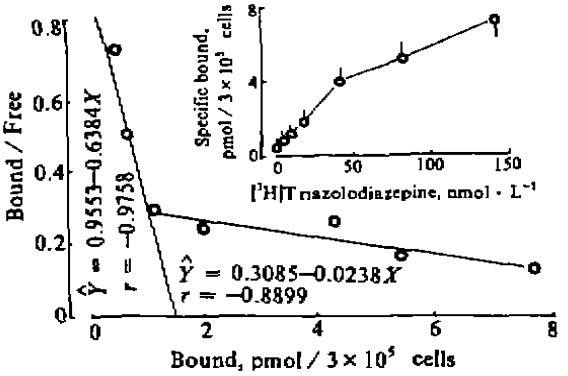


Fig 3. Displacement of [³H]triazolodiazepine binding to intact CMEC by C_{16}–PAF and SZ–1. $n=3$, $\bar{x} \pm s$.

DISCUSSION

Many experiments suggested that the cerebral vascular disorders might be related to the PAF[7,8,10]. The data reported here described for the first time the characterization of PAF receptor on CMEC using [³H]triazolodiazepine. The binding of [³H]triazolodiazepine to CMEC was specific, reversible, and saturable. Scatchard analysis indicated that the existence of 2 specific binding sites on CMEC is different from that of other cells such as platelets, neutrophils exhibiting only one kind of the sites[11,12]. This suggested that the importance of PAF in the cerebrovascular diseases. The action of PAF in the cerebrovascular system might be mediated

through the PAF receptor.

SZ-1, a new synthetic compound, not only displaced the [^{3}H]triazolodiazepine binding to CMEC, but inhibited the aggregation of washed rabbit platelets induced by PAF *in vitro*, the release of [^{14}C]arachidonic acid in CMEC, and the Evans blue staining in rat brain induced by PAF (data not shown). It suggested that SZ-1 is an antagonist of PAF receptor.

Specific binding sites of PAF have been found in various cells. But binding studies with [^{3}H]PAF remain somewhat difficult because of the high level of nonspecific binding, the inconsistent binding and uptake of the radioligand. Thus, we made use of the PAF receptor antagonist [^{3}H]triazolodiazepine as radioligand to investigate the PAF specific binding sites on the intact bovine CMEC for decreasing the nonspecific binding.

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培养牛脑微血管内皮细胞上血小板激活因子的特异结合部位及药物的拮抗作用

R966

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提要 在体外培养的小牛脑微血管内皮细胞上, 用 PAF 受体拮抗剂[^{3}H]triazolodiazepine 研究 PAF 受体. 结果表明[^{3}H]triazolodiazepine 在该细胞上有两个特异结合位点, 其 K_{d1}, K_{d2}, B_{max1} 和 B_{max2} 分别为 3.13 nmol · L^{-1}, 83.96 nmol · L^{-1}, 1.50 pmol / 3 × 10^5 cells, 和 12.96 pmol / 3 × 10^5 cells. 而且[^{3}H]triazolodiazepine 与内皮细胞的结合可被 C_{16}-PAF 和 SZ-1 所取代. 提示脑微血管内皮细胞上有 PAF 特异结合部位.

关键词 血小板激活因子; 血管内皮; 药物受体